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C. H. WADDINGTON

ORGANISERS AND GENES

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THE EPIGENETIC LANDSCAPE

From a drawing by JOHN PIPER

Looking down the main valley towards the sea. As the river flows away into the mountains it passes a hanging valley, and then two branch valleys, on its left bank. In the distance the sides of the valleys are steeper and more canyon-like. (See p. 91.)

ORGANISERS & GENES

by

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1947

JUSTIN
406 So. ~~Chester~~

P R E F A C E

This short discussion is not intended as a general survey of the whole field of experimental embryology. It is an account of the lines of thought suggested by a single set of phenomena, those of embryonic induction. I have attempted to approach this particular aspect of development from as many angles as possible; and it is fortunate that the process of induction is not only one which lies open to causal analysis, but is also one which impinges on nearly all the fundamental problems of development. It is naturally important to enquire how generally one can apply the principles derived from the study of so important a phenomenon. I have not, however, attempted any extended discussion of the relation between the inductive mechanisms of vertebrates and the causal processes which have been discovered in embryological investigations of invertebrates. I have instead devoted some space to pointing out the similarities between the concepts derived from the consideration of the organiser and those which arise in connection with the developmental effects of genes. The two sets of phenomena have of course been investigated in different organisms, but the principles of genetics are so uniform throughout the animal kingdom that it may not be too much to hope that processes occurring in one group may provide a valuable guide to those in another.

It should perhaps be pointed out that throughout the discussion I have tried to adopt an inductive approach. Thus in discussing induction we find evidence that there are two aspects to the matter, which have been called evocation and individuation. It will avoid confusion if it is remembered that these are essentially names for two subjects of investigation rather than for two ultimately different principles of explanation. In fact, the later discussion shows that some at least of the problems of individuation can probably be solved in the same terms as those of evocation.

It is a consequence of the same method of approach that the discussion of the most abstract concepts, such as fields and organisation, comes at the end of the book rather than at the beginning.

I should like to thank G. Bateson and Dr J. Holtfreter, who have read several of the chapters and discussed them with me; and I am also grateful to John Piper for his interpretation of my somewhat romantic conceit, the epigenetic landscape. My greatest obligation, however, is to Dr Joseph Needham, who has been my close collaborator during most of this book's gestation.

C. H. W

Pasadena and Cambridge

1939

CONTENTS

	PAGE
PREFACE	vii
CHAPTER I. THE CAUSAL ANALYSIS OF DEVELOPMENT	1
CHAPTER II. ORGANISERS IN DIFFERENT CLASSES OF VERTEBRATES Amphibia—Fish—Birds—Mammals.	5
CHAPTER III. THE ANALYSIS OF ORGANISER ACTION	14
CHAPTER IV. EVOCATION The dead organiser—The specificity of the evocator—The nature of the evocator—The activation of the evocator.	20
CHAPTER V. COMPETENCE The origin of competence—The nature of competence—The loss of competence—The evocator-competence reaction—The genetic control of the evocator-competence reaction.	41
CHAPTER VI. GENIC ACTION The nature of the substances produced by the genes—Gene effects—Substance and pattern—The localisation of gene effects—The interaction of gene effects.	56
CHAPTER VII. THE TEMPORAL COURSE OF GENE REACTIONS Time-effect curves—Developmental stages—The branching-track system—The epigenetic landscape.	69
CHAPTER VIII. INDIVIDUATION Individuation and evocation—The structure of the egg—Local chemical differences—Regional induction.	94
CHAPTER IX. MORPHOGENETIC MOVEMENTS	106
CHAPTER X. ORGANISERS AND GROWTH The determination of relative growth rates—The individuation of growth.	117

too often find ourselves forced to accept the natural separation of the biological disciplines into the two great groups we have mentioned; the synchronic or physiological, and the diachronic or developmental; or the cyclic processes, repeatable in one life-history, and the progressive, which an individual undergoes only once. Bridges between these two fields already exist, though in somewhat skeleton form; the study of the chemical nature of the gene, and its mode of action, as well as the physiological and biochemical study of development, are beginning to bring the concepts of the two types of biology into contact.¹

In this book I shall not be primarily concerned to discuss the relations between synchronic and diachronic biology, although some aspects of this question will be touched on. My first aim, however, is to contribute to the bridging of a narrower gap, which exists between the two fields of embryology and genetics within the general sphere of diachronic biology.

The causal analysis of development on the whole lags behind that of the day-to-day functioning of organisms. It is true that the elements of the theory of development on the largest time-scale, that is to say, the theory of evolution, was one of the earliest biological theories to be enunciated in a satisfactory form; but its elaboration has been very slow, and only in the last few years have the theoretical researches of Wright, Fisher, Haldane and Darlington, and the practical work of Tchetverikov, Sturtevant, Dobzhansky and others, carried our understanding much beyond the point reached by Darwin. On the intermediate time-scale of genetics, much more progress has been made, and this is one of the most fully developed of all biological sciences. But in the short end of the range, again, the causal study of development has also advanced only very slowly.

The older investigations of embryonic development led to results of two different kinds, which were not only apparently diametrically opposed to one another, but were each of them such that they offered no obvious hope for further insight into the processes involved. On the one hand, it was found that, in many eggs, each part was capable of forming a certain part of the adult, and that part only. The egg was a mosaic of regions, each with a definite potency for development. No causal mechanism could be discovered; the eggs just developed, and the parts just developed,

¹ Waddington, 1939*a*.

and that was all that could be found out. On the other hand, eggs of certain other species showed the contrary behaviour; any part of the egg could, it seemed (though this is now considered very doubtful), become a whole embryo. Again, no causal mechanism appeared; and in fact some biologists, such as Driesch, gave up hope of discovering a material causal mechanism which could explain the facts.

Attacking the problem indirectly through a study of heredity, genetics in the early years of the century did indeed succeed in revealing some true causal antecedents of adult structures and functions. The method could, by its very nature, only show that genes are responsible for the development of characters which may be different in two organisms which can nevertheless breed together. These characters are all dependent on nuclear factors; the properties of the egg cytoplasm are not susceptible of analysis by the same method. Even if we assume, as we are probably justified in doing, that these cytoplasmic properties are themselves dependent on the genetic endowment of the mother by whose body the eggs are formed, that assumption, important though it may be in connection with the theory of evolution, is not relevant when we are considering the mechanism of development during a single individual life. Moreover, the discovery of genetic factors reveals only the first link of a chain of causal events, whose other end, the adult character, is known, but whose intermediate links require elucidation. The genes cannot be regarded as immediately effective in causing the successive processes of differentiation, although they are undoubtedly the fundamental elements which ultimately control them. A coherent theory of development cannot be founded on the known properties of genes; in fact, it seems much more hopeful to try to fit our somewhat scanty knowledge of the developmental actions of genes into a framework founded in the first instance on the direct experimental study of development.

The essential question for a theory of development is this: What is the immediate causal network underlying this particular process of differentiation occurring at this particular time? The first partial answer to such a question was given by Spemann.¹ His success was partly due to the elaboration of an adequate technique of operation. But it was also dependent on the clear formulation

¹ Spemann, 1918; Spemann & Mangold, 1924.

of the essential query just mentioned. It was known that in the amphibian gastrula the dorsal half of the animal hemisphere develops into neural tissue, the ventral half into epidermis. By a series of experiments which have become classical, Spemann showed that the reason for this difference in behaviour lies in the fact that the dorsal region comes in contact with the tissues which are invaginated to become mesoderm, and that the neural differentiation is a response to a stimulus emanating from the mesoderm.

The principle that the differentiation of a certain tissue or organ may be induced by a stimulus exerted by some other part of the egg had been adumbrated by Roux in his idea of dependent differentiation, and was partly confirmed by Spemann's earlier researches on the interaction between the eye-cup and lens. But it was the work on the neural plate, which is the first-formed and primary organ of the vertebrate body, which demonstrated the importance and scope of the mechanism. The causal analysis of development may be said to have first started with this discovery. At the same time, it is obvious that we have only a beginning of an answer to our essential query. No "stimulus", nor single cause, is itself an adequate explanation of anything. We must hope eventually to know the whole complex system of actions and interactions which constitute the differentiation.

The significance, and at the same time the crudity, of the ideas involved in Spemann's discovery may perhaps best be appreciated by an analogy. It has been known for some hundreds of years that the "cause" of muscular contraction is stimulation by a nerve. The statement that the "cause" of an embryonic differentiation is stimulation by an organiser is just as basic, in its own sphere, and just as crude. Both statements obviously require, and at the same time provide a guide for, further investigations into the nature of the stimulus and the nature and mechanism of the response.

CHAPTER II

ORGANISERS IN DIFFERENT CLASSES OF VERTEBRATES

BEFORE entering on the discussion of the mode of action of organisers, it seems advisable to summarise shortly what is known about the organisers of the various groups of vertebrates.

Amphibia.

The main facts about the positions and functions of the amphibian organisers, on which Spemann¹ did his classical work, are well known and have recently been reviewed in several publications. The first to become active during development is known as the primary organiser, and lies anterior and dorsal to the blastopore in the early gastrula; it extends laterally for a considerable distance on each side of the dorsal midline; in fact, Holtfreter² has recently shown that in some respects even the most ventral tissue of the marginal zone must be considered to belong to it.

The activity of the organiser is most conclusively demonstrated by grafting experiments. On being inserted into a new region of the gastrula, a fragment of tissue from the primary organiser pursues its own characteristic development, invaginating into the interior of the embryo and developing into mesodermal tissues such as notochord and somites. Some slight change of developmental fate may be involved in this differentiation (p. 100) and part of the organiser tissue may develop atypically into neural tissue. A much more profound change in developmental fate is, however, produced in the neighbouring ectoderm; even if the graft has been made into a region where the ectoderm would normally develop into epidermis, the organiser stimulus causes it to differentiate into neural tissue. The induced neural tissue and the mesodermal structures from the graft often become adjusted to one another so as to form a comparatively normal embryonic axis; and a similar inducing action on the endoderm may provide this embryonic rudiment with an appropriate gut.

¹ Spemann, 1938.

² Holtfreter, 1936, 1938a.

The primary organiser is succeeded by a series of others, which may be spoken of as secondary and tertiary organisers. Of these perhaps the best known example is the eye-cup, which induces the formation of the lens from the ectoderm with which it comes in contact. The other placodes, of the ear and nose, for example, are also dependent on inducing stimuli, which may in some cases proceed simultaneously from several different organiser regions; Holtfreter¹ has spoken of the ear as being formed under a cross-fire of organisers. Similarly, induction phenomena have been demonstrated in the development of many of the later formed embryonic organs, such as the mouth, in the formation of organs during metamorphosis and probably in regeneration in the adult.

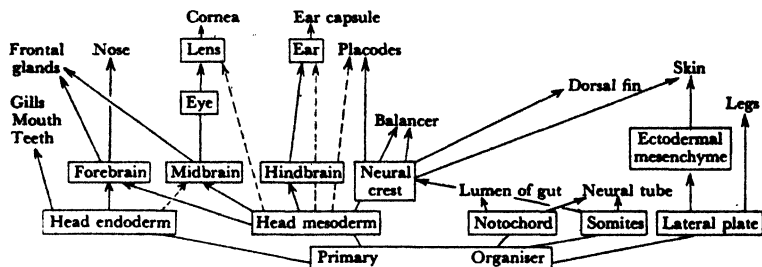


Fig. 1. Organisers in the Amphibia. The main organiser regions are enclosed in rectangles. The lines join organs which are developed one from the other, while the arrows show inducing relations, the dotted ones being of less importance. (After Holtfreter.)

Holtfreter² has recently summarised our knowledge of the succession of primary, secondary, tertiary, etc. organisers in Amphibia in a diagram which is reproduced, with some minor alterations, in Fig. 1. In this diagram, the most important inducing influences are indicated by full arrows, less powerful ones by dotted arrows; developmental forms of the same mass of tissue (e.g. the eye which is formed out of the midbrain) are connected by a line without an arrow.

Fish.

The gastrula of cyclostomes is extremely similar in general conformation to that of the newt. Weissenberg³ has given an exact description of the location of the presumptive regions of the lamprey and shown that their arrangement follows very closely

¹ Holtfreter, 1935 a.

² Holtfreter, 1938 a.

³ Weissenberg, 1934.

those described by Vogt in *Triton*, the main difference being that in the cyclostomes the presumptive mesoderm does not extend completely round the gastrula, but is absent on the ventral side. Bytinski-Salz¹ has repeated many of the classical amphibian experiments with this material, and succeeded in proving that here also the organiser is located just dorsal to the blastopore.

In the meroblastic eggs of teleosts, the conditions for gastrulation are of course very unlike those in the Amphibia or Cyclostomes, and the distribution of presumptive areas is modified accordingly.² Workers in America and Germany³ simultaneously succeeded in overcoming the considerable technical difficulties of the material, and discovered that here again the blastopore is not only the centre of gastrulation movements but also the site of an inductive agency. The direct demonstration of the organiser was made by grafting the invaginating meso-endoderm under a new region of the blastodisc; the graft continued its normal development into chorda, somites and gut endoderm, and at the same time induced the formation of a neural plate by the overlying ectoderm. Some of the inductions obtained are extremely complete, except for slight deficiencies in the head region.

Birds.

After their discovery in Amphibia, organisers were next detected in birds.⁴ The phenomena in this group are probably not so widely known as those in Amphibia, and have not been recently summarised, and therefore require slightly fuller treatment.

The organisation centre in the Amphibia and fish is situated at the focus of the gastrulation movements by which the endoderm and mesoderm are brought to their final positions under the ectoderm. In the chick, these movements take place in two phases; the endoderm is formed at about the time of laying, while the mesoderm is not formed till the primitive streak stage some hours later. The exact mechanisms involved in these processes are still under dispute, and until certainty has been reached about them, some doubt must remain as to the positions of the presumptive endoderm and mesoderm before the invagination occurs. Until recently the standard account of endoderm formation was that of Patterson, who stated that, in the pigeon, the posterior edge of

¹ Bytinski-Salz, 1937 *a, b*; Yamada, 1938 *a*.

² Pasteels, 1936; Oppenheimer, 1936 *a*.

³ Luther, 1935; Oppenheimer, 1934 *a, b*, 1936 *b*.

⁴ Waddington, 1930.

the blastodisc turns underneath and grows forward as the lower layer. The careful investigations of Jacobson¹ have, however, shown that the endoderm is formed from a region just anterior to the posterior edge. Thus the presumptive endoderm, before its invagination, lies in the posterior part of the circular blastodisc, but its exact extent is not known.

The mesoderm is formed from the primitive streak, which appears as a thickened ridge in the posterior part of the blastoderm shortly after endoderm formation has been completed. The mesoderm, during its formation, is carried from the upper layer (epiblast) into the space between the epiblast and the endoderm; it can therefore be said to be invaginated.²

In the stage with a fully formed streak, which is the stage with which the experimental work has been mainly concerned, there is general agreement that the entire axial structures of the embryo (notochord, somites, neural tube) are concentrated round the anterior two-thirds of the streak, with the mesoderm centrally placed. The most peripheral of the definitive mesoderm is invaginated first through the primitive streak and is therefore most centrally placed, while the last-invaginated material, the notochord, is on the lateral boundaries of the mesoderm arc. Outside the arc of mesoderm is the presumptive neural material, also in the form of an arc.

Speculations as to the position of the organisation centre of the chick embryo were made in the early days of Spemann's discovery. Gräper argued that in the chick it is the formation of the endoderm which must be regarded as homologous with invagination in the Amphibia and that one would therefore expect the organisation centre to be located at the posterior edge of the unincubated blastodisc. Wetzel,³ on the other hand, regarded the primitive streak, in particular its anterior end or Hensen's node, as the true homologue of the blastopore, and at one time suggested that it was the organisation centre. A decision between these possibilities, or even a demonstration that either of them is true, awaited the discovery of a technique of operating on the bird embryo.

The earliest technique to be employed was that of chorio-allantoic grafting; in this method fragments of tissue are isolated

¹ Jacobson, 1938.

² Gräper, 1929; Jacobson, 1938; Pasteels, 1937*a*; Wetzel, 1929*a*.

³ Wetzel, 1924; cf. 1929*b*.

on the highly vascular chorio-allantois of older embryos, where they become invaded by blood vessels which supply them with oxygen and nourishment. The method should allow one to study the capacity for independent differentiation of isolated fragments, but it is open to many criticisms, both on the score of its theoretical validity and of the way in which it has been actually used.¹ The theoretical criticism is that the circulating blood contains a substance capable of inducing neural tissue in *Triton*, and probably in the chick; one cannot therefore expect it to be a "neutral" situation, and the fact that it is not one is shown by the incomplete development of whole blastoderms when isolated upon it. The practical restriction on the use of the method lies in the difficulty of isolating suitable fragments of tissue. All authors using the method, until recently, have isolated fragments containing, as well as ectoderm, either endoderm or mesoderm or both, that is to say, containing inducing tissues. Thus in the early days of such investigations, Hoadley² obtained neural differentiation from isolated fragments from pre-primitive streak stages and drew the unjustified conclusion that the determination of neural tissue had already occurred.

Recently Rudnick³ has obtained neural tissue from fragments of blastoderm which were isolated from endoderm, and dissected before the invagination of mesoderm in such a way that they should not have included any presumptive mesodermal tissues. Abercrombie (unpublished) has evidence to the same effect. The development of neural tissue in these fragments may indicate a real precocious tendency for this type of differentiation, present before and independently of any action of the mesoderm organiser. Such a tendency has frequently been invoked in the Amphibia, but the possibility of it was finally banished by Holtfreter's⁴ exogastrulation experiments. In chick material on which the endoderm organiser has already been acting, a similar tendency would perhaps not be too unexpected.

Hoadley was also able to draw an entirely correct conclusion, namely that Hensen's node, which had not been included in some of his grafts, is not essential to the formation of the embryo. This result was confirmed by Wetzel,⁵ who obtained differentiation of

¹ Waddington, 1935*a*.

³ Rudnick, 1938*b*.

⁵ Wetzel, 1929*b*.

² Hoadley, 1926, 1927.

⁴ Holtfreter, 1933*c*.

neural tissue from the posterior parts of blastoderms which he sectioned *in ovo*. The difficulties offered to exact work by the shell and albumen, made it impossible to proceed much farther with this technique. However, the technique of tissue culture had by this time reached a stage at which it was immediately possible to explant young blastoderms, removed from the egg, and keep them alive in culture long enough for the development of the main axial organs. The explanted embryos are easily operated upon.

The position of endoderm invagination cannot be determined by inspection of the living blastoderm until endoderm formation is nearly complete and the primitive streak is beginning to appear. At this stage the endoderm may be removed and replaced in a different position. The orientation of the endoderm is found to have an influence on the direction in which the primitive streak grows; the streak always tends to elongate towards the region under which lies the anterior end of the endoderm¹. This pointed to an inducing action of the endoderm, which was subsequently demonstrated; if the endoderm is reversed, so that its anterior end lies under the primitive streak, a new primitive streak may be induced above its posterior end, so that two embryos are developed on the blastoderm, pointing in opposite directions.² Dalton³ argues that this inducing action of the endoderm is normally active even in young primitive streak stages, since he found no differentiation of structures characteristic of the axial mesoderm in chorio-allantoic grafts of the posterior parts of young primitive streaks from which the endoderm had been removed, but this result is rendered somewhat uncertain by the fact that Dalton judged the presence of axial mesoderm only by the presence of its specialised derivatives such as mesonephros. Twiesselmann⁴ reports the production of double monsters, apparently by splitting of an organisation centre, when electrolytic injuries are made slightly anterior to the posterior edge of the unincubated blastoderm; it is possible that his injuries affected the endoderm organiser, although he himself suggests that they affected the future primitive streak material. Butler⁵ grafted fragments of the unincubated blastoderm on to the chorio-allantois and obtained satisfactory development only from those pieces which included

¹ Waddington, 1930, 1932.

³ Dalton, 1935.

⁵ Butler, 1935.

² Waddington, 1933 *b*.

⁴ Twiesselmann, 1938.

the posterior segment of the blastoderm, which may be taken as supporting the suggestion that there is an organisation centre in this region.

There is thus good evidence that, as Gräper suggested, the formation of endoderm is closely connected with organisation phenomena. The structure which arises in response to the organising stimulus is the primitive streak, and from the streak a new organising stimulus is exerted on the rest of the epiblast. The proof that the streak is an organisation centre was also first given with the help of the *in vitro* technique;¹ it was shown that if two epiblasts were placed with their mesoderm faces together, each primitive streak could induce the formation of a neural plate, and probably an entire embryonic axis, in the part of the second epiblast against which it lay. Induction can also be performed by small pieces of streak grafted between the epiblast and endoderm of a host embryo.

Suggestions that part of the streak (the most anterior part, or Hensen's node) was an organisation centre also came from another quarter, namely from authors who claimed that differentiation could only be obtained from such isolated fragments of blastoderm as include Hensen's node. Wetzel² was the first to use this supposed fact as an argument to prove that Hensen's node is an organisation centre, but he later showed that in point of fact parts of the blastoderm not including Hensen's node are capable of further development, and he therefore withdrew his assertion.³ It has, however, been repeated by most of the chorio-allantoic workers other than Hoadley, though Waddington, and more recently Wetzel, Waterman and Dalton,⁴ using the chorio-allantoic technique, confirmed the fact that Hensen's node is not essential for development. It is to be noticed also, that even in the works of those who believe in the indispensability of the node, the size of the "node" (including the so-called "node field") grows larger as time goes on, and it becomes clear that less and less of the anterior part of the streak can be regarded as essential.⁵ In any case, even if the node were essential for development, that would not prove it to be an organiser in Spemann's sense, which is defined by its capacity for altering the course of development of tissue on which it acts.

¹ Waddington, 1930, 1932.

² Wetzel, 1924.

³ Wetzel, 1929 *b*.

⁴ Dalton, 1935; Waterman, 1936; Wetzel, 1936.

⁵ Rawles, 1936; Rudnick, 1938 *a*.

The *in vitro* experiments have shown that the inducing capacity is present in at least the anterior two-thirds of the streak, which is the region in which the presumptive axial mesoderm is located; it is still uncertain whether there is any inducing capacity in the posterior third of the streak, which seems to be made up entirely of presumptive peripheral mesoderm. The inducing capacity belongs to the mesoderm, although it can also be shown by neural tissue (homoigenetic induction). It appears to be rapidly lost from the differentiated notochord, but this appearance may be illusory, as it is probable that very close contact is necessary for

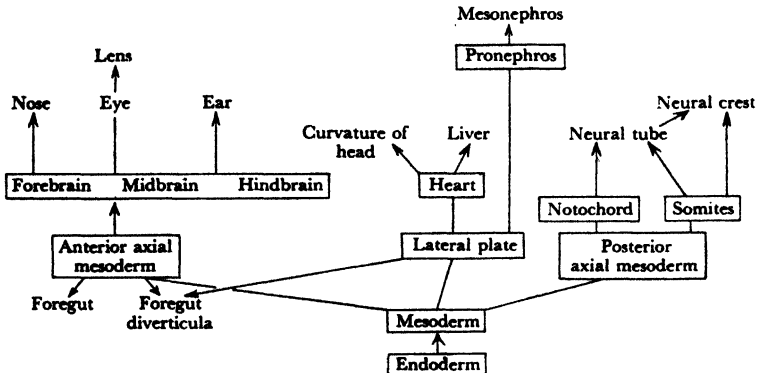


Fig. 2. Organisers in the chick. Conventions as in Fig. 1.

the spread of the inducing agent in the chick, and such a degree of contact is not attained between the ectoderm and the compact notochord.

A certain number of secondary and tertiary organisers are known in the chick, though this form is not nearly so well worked out as *Triton*. A diagram of most of the known organiser relations in early development is given in Fig. 2.

Mammals.

Workers on the mammalian embryo have used the same methods as are employed on the chick; isolation in other animal sites, and *in vitro* cultivation. The isolation sites (chorio-allantois of the chick, omental bursa, kidney capsule, etc. of the rabbit) have none of them proved very satisfactory and have as yet only been used for isolation from stages older than the primitive streak stage. The isolates have shown essentially mosaic development with some

resorption and inhibition of differentiation. By *in vitro* cultivation embryos of the rat or rabbit in the primitive streak stage can be kept alive long enough to form well-recognisable embryonic axes. Isolated fragments of the primitive streak, grafted into other sites in the embryonic shield, can differentiate to neural tissue even in the absence of Hensen's node, which is thus not essential for differentiation in this form any more than in the chick.¹ It has also been shown that the embryonic axis of the one-somite rabbit has an organising capacity, since it can induce neural tissue when grafted into a chick embryo.² Grafts of rabbit streak into rabbit embryonic shields have never yet produced inductions, probably for reasons connected with the technical difficulties of performing the operations. But the competence of the rabbit ectoderm to react to inducing stimuli has been demonstrated by the successful induction of neural tissue by grafts from the chick primitive streak into the rabbit shield of the streak stage³.

Törö,⁴ working with the rat embryo grown *in vitro*, has recently been successful in producing homoplastic inductions, by grafts of rat primitive streak into other rat embryos. His results, however, are peculiar in several respects, particularly in the fact that inductions are reported after merely placing tissues into the amniotic cavity. In no other group has it been shown that the organiser stimulus can be effective when applied to the external surface of the ectoderm. In some cases also the degree of differentiation attained by the induced tissues is perhaps hardly sufficient for one to be quite confident of discriminating between neural tissue and thickened ectoderm. But although these criticisms suggest that further work is urgently needed, there can be little doubt of the main point at issue, which is that the embryonic development of the mammal is also brought about by the action of organisers.

The evidence cited above relates to the organiser action of the primitive streak or tissue derived from it. Nothing definite is known of the movements going on in the mammalian streak, but one can hardly be wrong in assuming that some kind of invagination of mesoderm occurs there. The mode of origin of the endoderm is still more problematical, and there is no information as to its organising function, if any.

¹ Jolly & Lieure, 1938; Nicholas & Rudnick, 1937.

² Waddington, 1936*a*, 1937.

³ Waddington, 1934*c*.

⁴ Törö, 1938.

CHAPTER III

THE ANALYSIS OF ORGANISER ACTION

THE organiser was originally looked for and found as the agent which decides between the alternatives of neural and epidermal differentiation of the ectoderm. Its functions, however, are actually a good deal more complicated than this, as Spemann realised when he gave it its name. The word organiser clearly predicates some ordering or arranging function, which is not at all implicit in the problem which was originally posed. Some authors, in fact, seem to feel that the word implies powers which cannot reasonably be accommodated within a material or scientific framework, and therefore propose substituting for it such timid names as "neural inductor" or the like. The actual properties of the organiser cannot, however, be eliminated from consideration merely by refusing to refer to them in its name. Organiser is the term originally proposed for the inductive region and is already sanctioned by extensive use. The word need not carry into science any unwanted connotations there may be in its everyday usage; its biological meaning is just exactly those properties which we actually demonstrate in it and no more.

The functions which make it proper to speak of an organiser rather than of a mere inductor were most clearly demonstrated by Spemann in a paper published in 1931.¹ At that time he brought together a great deal of evidence in support of the two following theses. Firstly, the organiser induces not neural tissue, but a neural organ which may be complete or partial, but even in the latter case can be seen to represent some part of the normal neural system (brain, spinal cord, tail, etc.); the regional character of the induced organ may be further exhibited by the secondary organising functions it assumes, an eye inducing a lens, a fore-brain inducing nasal placodes, etc. Secondly, different parts of the organiser have different properties in respect of the region of the neural system which they induce. Thus the first-invaginates mesoderm, which will normally move so as to lie under the anterior part of the embryo, tends to induce anterior structures such as

¹. Spemann, 1931 a.

brain, eye, etc., while the later-invaginated, presumptively posterior, material tends to induce more posterior regions such as the spinal cord and tail. These potencies for "regional determination" are, however, only labilely fixed. In the first place, a grafted organiser seems always to induce a rather larger region of the body than might have been expected from its presumptive fate. In the second place, Spemann showed that the regional character of an induced neural plate is influenced not only by the nature of the organiser inducing it but also by its location within the body of the host. This influence of the host, as Waddington and Schmidt¹ pointed out, affects not only the induced neural plate but also the grafted organiser. If, for example, a presumptively posterior organiser is grafted near the anterior end of an embryo, not only is the neural plate which it induces usually caused, by the influence of the host, to form as a brain, but the development of the grafted mesoderm is also affected so that it becomes head mesoderm.

Spemann apparently considered the faculty for regional determination as an essential part of the power of induction. Waddington and Schmidt, working on the bird embryo, found evidence that the association of these two functions is not indissoluble. An organiser need not be either a head or a tail organiser, or correspond to any definite section of the body. They noticed that effects on regional character are always assimilative; a host embryo tends to cause a graft and its associated induced structures to conform to the region in which it lies; or a graft induces a neural organ conformable to its own regional character. In the chick this is particularly striking, since grafts near the host primitive streak form organs which agree exactly with those of the host at the same level, or may even fuse completely with them, while if the graft is made some distance from the streak, the influence of the host is much weaker, and the regional character of the induction is dependent only on that of the graft.

Now this assimilative character is not necessarily associated with induction. If the endoderm is stripped off a chick blastoderm, the mesoderm and ectoderm are left intact, and the mesoderm is fully provided with all the appropriate ectodermal structures; these structures do in fact develop fairly normally. But the mesoderm can none the less induce a further neural plate if it is brought

¹ Waddington & Schmidt, 1933.

in contact with more ectoderm; and this induced plate is "extra" and cannot be considered in any way assimilative.¹ The same thing is true in Amphibia. The endoderm can be entirely removed from the young gastrula of *Discoglossus*. The remaining mesoderm and endoderm differentiates into a tadpole which is quite well formed, considering the mechanical conditions. But the lower side of the invaginating mesoderm now comes in contact with the ventral ectoderm, from which it is usually separated by the mass of endoderm, and in this ventral ectoderm an extra neural plate is induced.² Similarly, Schechtman³ has explained some double formations in Anura by the hypothesis that the mere pressing of the blastocoel roof against the not yet invaginated mesoderm leads to the induction of accessory structures.

This type of non-assimilative induction was at first spoken of as "induction-as-such"; later the word "evocation" was used for it.⁴ The assimilative induction which is concerned with the regional character of the induced structures was called "individuation".

The distinction between these two aspects of induction can be deduced in another way. If we make an organiser graft, the place where the induced neural plate appears is dependent solely on the graft and is independent of any influences proceeding from the host embryo, but on the other hand the regional character of the induction is influenced by the host as well as the graft. The processes of producing an induction and determining its regional character present two different problems, since an influence which affects one has no influence on the other. The first is evocation, the second individuation. Much of the later discussion will be devoted to an attempt to analyse and clarify these two concepts.

Meanwhile it is necessary to draw attention to the other side of the reaction of induction. The organiser does not by any means impose the whole process of differentiation on completely passive and indifferent material. On the contrary, the embryonic ectoderm is only capable of responding to the organiser stimulus during a certain period, and during that time, as will be discussed more fully later on, it has certain capacities for development which play a part, perhaps even the major part, in determining the actual course of differentiation.

¹ Waddington, 1932; Abercrombie, 1937.

² Waddington & Taylor (unpubl.).

³ Schechtman, 1937.

⁴ Needham, Waddington & Needham, 1934.

The faculty of response to an organiser is known in German as *Reaktionsfähigkeit*; in English, I have proposed the name *competence* for it.¹ The time during which a tissue is capable of reacting to an organiser stimulus is the period of competence in respect to that organiser. As this period proceeds, it is often found that the reacting tissue gradually acquires the power to continue its development into the induced organ after removal of the organiser; the variation in strength of the potentiality usually expresses itself as an increase in the percentage of isolated fragments which can continue the induced type of development. Such a tendency, definite but not fixed, to develop in a certain direction is referred to as a labile determination, or, in the German phrase, as a *Bahnung*. It is, in a sense, the reciprocal of competence; as the tissue acquires a stronger and stronger tendency to develop in a certain direction, it loses its competence to react to an organiser which would induce it to develop in some other way. Eventually a state is reached in which a certain type of development is definitely prescribed; if pieces of the reacting tissue are removed from the sphere of action of the organiser and submitted to other conditions, 100 per cent of them develop into the induced organ, as though the organiser were still present. Such tissue can be spoken of as completely determined, or determined *tout court*.

The concept of determination is an absolute one; a tissue is determined to develop in a certain way when, in all circumstances in which it can develop at all, it does actually develop in that way. Being absolute, the concept can never be completely realised in practice. We can never subject a tissue to all possible conditions which will still allow it to develop. But we can test whether a tissue is determined relative to all influences which we suspect might alter its mode of differentiation. We can ask no more than that its determination passes this test; if there were any other influences with respect to which it is not determined they must lie beyond the horizon of our present ideas, or we should have tested them. For all practical purposes, then, it is perfectly possible to attach a definite meaning to the concept of determination.

A further ambiguity must, however, be cleared up before the concept can be considered as fully satisfactory. Harrison² has drawn attention to the fact that a tissue *A* may at one period in ontogeny show a strong tendency, amounting to determination

¹ Waddington, 1932.

² Harrison, 1933.

in the above sense, to develop into a given organ A' ; but at some other later period this organ A' may be able to undergo a new differentiation into some completely other organ B . For instance, in the early cleavage stages of Ascidians, the different regions of the embryo are strongly determined to develop in definite ways, but in the adult stage the organs which these regions have become are much more flexible; in regeneration, an organ can produce tissues which its embryonic primordium was quite unable to form. Harrison therefore argues that determination must be reversible, and that the term therefore loses much of its definiteness and value.

If determination is defined with reference to a certain end-product of development, Harrison's strictures undoubtedly hold, and determination must be considered as reversible. But this is to think in purely anatomical terms, as though all processes which lead to the differentiation of a certain tissue, for instance neural tissue, were the same. But surely it is probable that the processes undergone by the organ-forming regions of an Ascidian egg while they are forming some organ of the adult are very different from those by which that organ is formed from other adult tissues during regeneration. If determination is defined with reference to some process of differentiation, so that we say that a tissue is determined to carry out some process of development rather than that it is determined to form some organ, the cases of reversibility to which Harrison drew attention are no longer relevant. In fact the possibility of the reversal of determination would only arise if it could be shown that, after completing some process of development, a tissue could regress to exactly the condition it had been in before, and could then follow some totally different developmental course. No such demonstration has ever been given.

A remarkable example of the confusion which is caused by thinking of determination in anatomical terms, with reference to the future production of a structure, instead of in physico-chemical terms, with reference to the present occurrence of a process, can be taken from Spemann's¹ recent discussion of the term. He states that "We now generally follow the definition given by K. Hejder many years ago, according to which determination is the causation—the being caused as well as the having been caused—of the later fate of a part of the germ." But he proceeds as follows: "From the moment when a part of the germ thus

¹ Spemann, 1938.

contains within itself all the specific factors of its further development, or, in other words, when it is able to develop to its later destiny by self-differentiation (Roux) according to its prospective significance, we say it has become determined.¹ If therefore, in the two-celled stage of the frog's egg, the one lateral blastomere, after destruction of the other, is able to produce half a neurula, this blastomere has been determined for half formation." But surely this is just what we should not say, and in fact rarely do. It was indeed said by Brachet,² who was led thereby to deny the possibility of changes in developmental fate and of organiser action in stages later than the early cleavages of the frog's egg, a point in which he was clearly proved wrong by the work of Schotte³ and Schmidt.⁴

In point of fact, the capacity of a mass of tissue to produce, in the course of its development, some particular organ, very often gives no information whatsoever about the causation of the later fate of the part of the germ from which this organ has arisen. This becomes obvious if one changes the latter part of Spemann's statement quoted above so as to refer to another equally true fact: "If in the one-celled stage of the frog's egg, the cell is able to produce a whole neurula, this cell has been determined for whole formation." The statement in this form is absurd. It might be true of a highly mosaic egg, such as that of an Ascidian; it, precisely, is untrue in any important sense of an amphibian egg, from which we know that we can produce twins or double formations at a much later stage.

The production of a half-neurula by a half-blastomere in a frog's egg, to which Spemann refers, cannot be profitably discussed in causal terms if we do not consider the processes taking place between the isolation of the blastomere and the appearance of definitive organs. We know, in fact, that the important question is whether a reorganisation occurs in the structure of the grey crescent region which subsequently gives rise to the organiser. We can legitimately and profitably discuss the degree of determination of the blastomere in relation to this reorganisation, but not in relation to the later production, for instance, of one or two eyes.

¹ F. R. Lillie, 1929.

³ Schotte, 1930.

² Brachet, 1917.

⁴ Schmidt, 1933.

CHAPTER IV

EVOCAATION

The Dead Organiser.

As soon as it was discovered that an inducing stimulus is exerted by the invaginated mesoderm of the overlying ectoderm, it became tempting to consider the possibility that this stimulus was chemical in nature. But the facts regarding individuation, which were referred to in the last chapter, seemed to offer some difficulty to such a hypothesis. So long as the whole activity of the organiser was thought of as one indissoluble complex, it was difficult to see how it could be reduced to terms of a single chemical reaction. Some authors, in fact, were tempted to see in the organiser a last ditch in which the Drieschian entelechy could be entrenched. And Spemann and his school, not recognising any distinction between evocation and individuation, approached the possible chemical nature of the organiser with great caution. Marx¹ showed that the organiser was active, that is, would induce neural tissue, after narcotisation. Spemann² showed that its activity persisted after it had been crushed sufficiently to destroy the cell boundaries, but claimed, mistakenly as afterwards appeared, that no inductions could be obtained if the nuclei were ruptured.

It was not till the end of 1932 that it was unequivocally shown that the organiser would still induce after being killed. The discovery was announced jointly by Spemann, Bautzmann, Holtfreter and Mangold,³ working on the newt in Germany, and was immediately confirmed by Waddington,⁴ who had simultaneously and independently obtained evidence to the same effect in the chick.

Much the most successful of the German workers was Holtfreter, who reported inductions by tissue which had been killed by heating, freezing, drying or immersion in organic solvents. Spemann's successful cases were obtained with tissue killed by alcohol, Bautzmann's with heated tissue. Mangold reported an induction obtained by implanting a block of agar on which

¹ Marx, 1931.

² Spemann, 1931 *b*.

³ Spemann, Bautzmann, Holtfreter & Mangold, 1932.

⁴ Waddington, 1933 *a*, 1934 *b*.

inducing tissue had been laid; there was only a single success in the series of experiments, and in that the induction was small and not very satisfactory, so that the apparent evidence that the inducing substance can diffuse in a watery medium must be accepted with great caution; it is much to be desired that the experiment should be repeated. In Waddington's successful cases in the chick, the organiser tissue had been killed by heat.

In all these experiments the physical properties of the organiser tissue had been drastically altered by the killing agent, and it therefore became very difficult to suppose that the inducing stimulus was of a physical nature. Nothing but a chemical stimulus would fit the facts, and the way was cleared for an attack on the chemical nature of the inducing reaction. But before we discuss the chemical researches which have been carried out, it is necessary to emphasise that the process which these researches deal with is not the whole of induction, but is only that part of it which we have spoken of as evocation. This is immediately apparent when we remember that the living organiser has different parts with regionally different properties, while the implanted masses of purified substance are regionally homogeneous. The full discussion of this matter will be deferred to a later chapter (p. 95); here we shall merely note that the subject of the chemical work is an evocator substance, not an "organiser" substance.

A word must first be said about the technique of testing employed in the chemical work, and the criteria of success. It is of course impossible to use the classical grafting technique with non-living material. But if a pellet is inserted into the blastocoel of a young gastrula, it is usually carried forward by the invaginating tissues and pressed against the inner face of the ectoderm. When living organiser fragments are used in this way they induce in a high percentage of cases, and the method was used in the original experiments which proved the activity of dead organisers. Chemical substances to be tested are administered in the same way. It is first necessary to make them up into the form of small solid lumps, and this has usually been done by dissolving or emulsifying them in some suitable agent; agar jelly has frequently been used, and pure triglycerides have been employed, but the most useful method has proved to be emulsification in egg albumen which is subsequently coagulated by heat. It is probable, however, that the difficulty of diffusion of fat-soluble substances from such

jellies, or their degree of dispersion within the jelly, may be important factors in limiting the probability of an induction being produced. Dürken and Reith¹ have suggested that the partial or temporary inhibition of evocatory power which may be produced in the organiser by ultra-violet irradiation may be due to a reduction in the permeability of the cells to the evocator substance, which is therefore trapped within them.

Extensive tests have been made in which the carrier substances (agar, albumen, etc.) were implanted in a pure state. When any moderately hard substance is in contact with the inner surface of the ectoderm, some degree of proliferation is liable to occur, and these proliferations may sometimes be very large. Histologically, they consist of a mass of rather cuboidal epithelial cells, which are very different in appearance from typical neural tissue. Such an epithelial mass is clearly a negative result, giving no hint of neural evocation. The fully developed neural tissue of a successful induction is equally unambiguous; it forms a hollow tube, the walls of which are composed of a high columnar epithelium with elongated nuclei, and the superficial epidermis above it is often lifted up away from the neural tissue as a typical two-layered epidermis. These two easily characterised results are, however, the two poles of a rather incomplete series, and between them there are some intermediate types of tissue the interpretation of which is more doubtful.

In some cases, histologically normal neural tissue can be found which does not form a tube, and which does not seem to have been formed by the inrolling of a superficial neural groove but to have differentiated *in situ* from the lower part of the ectoderm. Holtfreter² showed that induction of this kind may occur even when living organisers are employed and that the induced tissues in later development form organs whose neural nature cannot be questioned; moreover, one can easily find cases in which a mass of induced tissue is a typical neural plate at one end and passes gradually into such concealed neural tissue at the other. The production of this tissue must therefore be considered a positive result and the tissue accepted as true neural tissue, in spite of the lack of orthodoxy in its origin. But the difficulty is to know where to draw the line in considering this type of evidence. The masses of tissue which, as we have just argued, must certainly be accepted

¹ Dürken, 1935; Reith, 1937.

² Holtfreter, 1933 *a*.

as neural, form plates several cells thick, and their ranks of elongated nuclei earned them the nickname "palisades". But much less definite palisades, usually only one cell thick, are often found at the base of a mass of proliferated ectoderm, forming a sheet of somewhat elongated cells lying immediately in contact with the implant.¹ These weak palisades ought not to be taken as the equivalent of neural tissue, and it is quite improper to refer to them as "neural plates".² But the question arises as to whether they always indicate some kind of evocating stimulus, however feeble. There is some evidence of this in the fact that they are more frequent and better developed in experimental series in which some implants have induced indubitable neural tissue. On the other hand, their frequency also depends to a large extent on the "reactivity" of the particular batch of eggs employed; those batches of eggs which show a higher proportion of successful inductions also show a high proportion of palisades. In the most reactive eggs, palisades may occur in response to the materials used as implant media, such as agar, albumen, etc. Clearly this makes it quite impossible to accept as positive any evidence derived entirely from palisades. But the occurrence of a marked increase in the frequency of palisades over that found in controls can often serve as a useful indication that further tests may produce valid inductions.

The investigation of the chemical nature of the evocator, using the methods which have just been discussed, was begun in the season immediately following the discovery of the activity of killed organisers. Several workers, including Holtfreter, Spemann, Fischer and Wehmeier, and Waddington, Needham and Needham, succeeded in preparing active cell-free extracts. These were, in the first place, crude watery extracts or centrifugates which were not sufficiently pure to indicate anything about the chemical nature of the active compound.

When more refined methods were used, different groups of workers obtained different results. The first suggestion was that of Fischer and Wehmeier,³ who thought that the evocator was glycogen. The activity of glycogen was, however, shown to be due to an impurity, and Waddington, Needham and their

¹ Cf. Wehmeier, 1934, Fig. 15.

² Barth & Graff, 1938.

³ Fischer & Wehmeier, 1933 a; cf. Woerdemann, 1933.

collaborators¹ reported that evocator activity could be found in the digitonin-precipitable and unsaponifiable fraction of the ether extract; and they also obtained inductions with two and possibly three different synthetic polycyclic hydrocarbons somewhat related to sterols. Fischer and his collaborators, however, while accepting the incorrectness of the original suggestion that the evocator is glycogen, and the fact that activity can be found in the ether extract, traced the active substance to the saponifiable fraction, and were able to obtain inductions with some synthetic higher fatty acids, muscle adenylic acid and nucleoprotein preparations.²

At this time both groups of workers attributed evocator activity to several different compounds, but in each case the compounds were thought to be chemically similar to each other; the Freiburg school attributed the activity to naturally occurring acids of fairly high molecular weight, while the Cambridge workers favoured sterol-like compounds. That the situation was really more complex was revealed when Waddington, Needham and Brachet³ showed that evocation can also be produced by methylene blue, a compound which cannot possibly be fitted into either of the categories under consideration up to that time, and which, moreover, is certainly not the substance to which the activity of the organiser is due in normal eggs. The attempts to identify the evocator must therefore proceed from the basis of the fact that evocation can be produced by compounds of several radically different kinds.

The Specificity of the Evocator.

The first question which must be discussed is whether there is such a thing as "the evocator". It is possible to draw an analogy with the investigations on artificial parthenogenesis, in which, as will be remembered, all attempts to identify a specific substance responsible for the activation of the egg in different techniques have failed; and the conclusion has been drawn that there is no single specific activating substance. Similarly, it might be suggested that the competence of the gastrula ectoderm was set on so fine a hair trigger that any of a number of stimuli are sufficient to touch it off.

¹ Needham, Waddington & Needham, 1934; Waddington, Needham *et al.* 1935 *a, b*, 1936 *b*.

² Fischer & Wehmeier *et al.* 1933 *b*, 1935.

³ Waddington, Needham & Brachet, 1936 *a*.

The first point to be noticed in connection with this suggestion is that the ectoderm, if it is on a hair trigger, can, unlike the egg, shoot in more than one direction. Appropriate stimuli, such as implants of albumen, or triglycerides containing certain sterols, can cause extensive proliferations devoid of neural tissue. Moreover, Chuang¹ was able, by the implantation of dead materials, to induce the ectoderm to develop into mesodermal tissues. There must therefore be some specificity in the chemical stimulus which causes neural differentiation. The question remains how much. It is fairly easy to imagine a system delicately balanced so that any of a large number of factors can turn it in one direction, any of another group in a second, and perhaps any of a still further group in a third. There is certainly no way of proving, from the facts which we know as yet, that such a picture does not apply to the ectoderm. But there are several reasons why the hypothesis is not a very attractive one in this connection.

We can, perhaps, reconcile ourselves to our failure to discover any specific substance responsible for the parthenogenetic activation of the egg, since we know that in the normal course of events this activation is performed by the sperm. But if we suppose that evocation can be produced by a non-specific stimulus, we are left entirely in the dark as to what happens in normal development. We know that some chemical stimulus is exerted by the mesoderm on the overlying ectoderm; and it is surely impossible to suppose that the stimulating substance is different in different eggs of the same species. There must then be a normal evocator substance; but if we adopt the hypothesis of an unspecific stimulus, we admit to no knowledge of what the normal substance is, and, as far as I can see, exclude any possible method of finding out.

Moreover, non-specific chemical stimuli do not seem to be at all commonly employed by animals in their physiological systems. It is difficult to think of any example of a stimulus which is normally chemical and which is at the same time non-specific. Carcinogenesis, indeed, can subvene on the stimuli exerted by several different chemicals, but it is difficult to take such a phenomenon as typical of a normal biological stimulus. For the initiation of development, the normal stimulus is not chemical; nor is it for the response of a nerve. Oestrus can be produced by several different compounds, but these seem to be not unrelated chemically, so

¹ Chuang, 1938.

that it is doubtful whether the stimulus can truly be called un-specific. Among plants, the auxin stimulus can be exerted by two very different groups of compounds, the true auxins and the indole-aliphatic acids; but this scarcely seems sufficient to justify calling the stimulus "unspecific". The auxin phenomena are of particular interest, since the response, like that of evocation, can also be produced by "unphysiological" stimuli of an un-specific kind, such as acids; but this is explained as a secondary effect of these stimuli on the "bound" auxin, already existing in the plant. It will be suggested later that an essentially similar explanation holds good for the evocatory action of acids and similar substances in amphibian development.

In artificial parthenogenesis, the activating stimulus, in so far as it can be characterised at all, seems to be one which produces a slight cytolysis of the egg surface. Some confusion has been caused by the fact that the phenomena involved in evocation may also include cytolysis, since this has been taken as a further support for a suggested parallelism between parthenogenesis and evocation.¹ But in the organiser reaction, cytolysis is involved at one step farther removed from the end-result, and, moreover, is involved in such a way as to make it apparently impossible ever to prove that it has a direct evocatory effect. Holtfreter² made the very important discovery that if non-evocating parts of the gastrula are cytolysed, or killed in almost any way, they become evocatory. Thus cytolysis confers the power of evocation on the cytolysed tissue, which is of course very different from what happens in parthenogenesis, where cytolysis seems to be the actual stimulus which brings about development. There is, in fact, no evidence that cytolysis can act as a direct stimulus to evocation; and it is very difficult to see how such evidence could be found, since in any case in which evocation and cytolysis were associated one could always argue that the cytolysis had affected some cells and conferred on them the power to evocate their uncytolysed neighbours.

Attempts to produce evocation by cytolysing agents do not give much support to the hypothesis that cytolysis may act in a direct way; while some inductions have been produced, they do not occur at all frequently, and are very small. Barth³ reports experiments with acids and alkalis and such cytolysing agents as

¹ Barth, 1939.

² Holtfreter, 1933*b*.

³ Barth & Graff, 1938.

digitonin. Only "palisades" were produced. As was stated above, such thickenings, taken alone, are insecure evidence of positive results, and this is particularly so when, as in this case, no evidence is presented as to comparable controls. Okada¹ has reported inductions produced by mechanical irritants such as silica, etc. which he interprets as due to a cytolytic action on some of the cells. Some of his figures are not very convincing, since they seem to represent cases in which the invaginating mesoderm of the host has been split by a mechanical obstacle and has therefore induced in two places. But others, such as his Fig. 4 (1938*a*), seem to be good evidence of true induction. But again, one must conclude that evocation is only rarely, and then rather feebly, successful. It is not at all clear why this should be so, if cytolysis is a direct evocating stimulus; techniques of artificial parthenogenesis depending on cytolysis are often successful in a high percentage of cases. The difficulty of producing evocation is much easier to understand if one supposes that the cytolytic agents act by the well-attested mechanism of killing some cells and thereby releasing an active evocator, since one is then faced with the necessity of striking a dosage which will kill enough cells without injuring their neighbours so much as to destroy their competence to react.

The Nature of the Evocator.

In seeking an alternative to the hypothesis of an unspecific stimulus, we have to consider the three phenomena of evocation by the living organiser, by the dead non-organiser and by various chemical substances. Of the pure chemical substances, only one, or rather only one group of chemically related substances, can be similar to the normal evocator which is responsible for the activity of the living organiser. The others must act secondarily. The simplest hypothesis of such a secondary action is that these substances cause the tissue on which they act itself to produce the normal evocator. One can account for the activity of dead non-organiser in a similar way; either the process of killing causes the production of the normal evocator, or some substance is formed which acts as a secondary evocator in the manner just mentioned.

A hypothesis of this kind, which postulates a single normal evocator acting directly and a number of other secondary evocators

¹ Okada, 1938*a*, *b*.

which act indirectly by causing the production of the former, has indeed the advantage that it explains all the phenomena without the need of postulating an unspecific stimulus, but it suffers from the disadvantage that it is difficult to prove. One can, however, point to several considerations which, if they do not by any means constitute proof of the hypothesis, at least make it appear likely. Thus there is considerable evidence that the normal evocator can

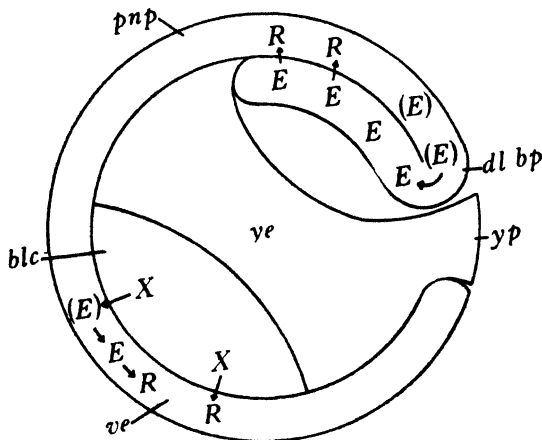


Fig. 3. Diagram to illustrate direct and indirect induction. At the upper right is the dorsal lip of the blastopore *dl bp*; here the masked evocator (*E*) is being liberated to form the active evocator *E*, which diffuses into the overlying presumptive neural plate *pnp* and produces the neural reaction *R*. Below at the left, a chemical substance *X* in the blastocoel cavity *blc* may either act directly on the ventral ectoderm *ve* to produce the neural reaction *R*, or it may act on the masked evocator (*E*), converting it to the free evocator *E*, which then produces *R*. (From Shen.)

easily be produced within the non-inducing gastrula ectoderm. For instance, Spemann and Geinitz¹ showed that if pieces of ectoderm are grafted into the region of the blastopore lip they become invaginated along with the mesoderm, and if then tested, are found to have acquired an ability to evocate. This is most easily interpreted to mean that the ectoderm has produced the evocator within itself. When one remembers that the non-organising ectoderm and the organising mesoderm are originally both parts of the blastocoel roof, and quite continuous with one another, separated

¹ Spemann & Geinitz, 1927.

only by the *Einstülpungsgrenze*, a line as purely formal as the earth's equator, then it seems very reasonable that one should be convertible into the other. But the demonstration is of course by no means secure. It is possible that the acquisition of evocating power by the invaginated ectoderm in Spemann and Geinitz' experiment is due simply to the diffusion of evocator from its surroundings and not to production within the ectoderm; though if this were so it is not clear why the graft does not merely differentiate into neural tissue. Similarly, the power of "homoiogetic" induction, which increases in the neural plate in proportion as the plate becomes determined, might be due either to the production of evocator within the neural tissue or to the diffusion of evocator into it; but again there is some reason to prefer the former hypothesis, since the evocatory power of the neural plate is so high that it is doubtful whether it can be fully accounted for by the hypothesis of diffusion.

The hypothesis of the activation of the evocator, to which we have been led by a consideration of the experimental data, is only a particular instance of a more general theory which we might have arrived at on *a priori* grounds. This theory is that, during the passage from competent ectoderm to determined neural tissue, a series of reactions is involved, in a complicated system of reactants, and that development may be switched into a neural channel both by affecting different steps of the reaction sequence, and also perhaps by affecting the concentration of different members of the set of reacting substances. The two steps in the sequence which we have so far discovered are the liberation or activation of the evocator and its action on the ectoderm after it has diffused into it. We do not know at all fully what the actual chemical reactions are in either of these steps, and we must consider the possibility that there may be several ways in which the evocator can be liberated; some of the evidence on this point will be considered later (p. 39). Moreover, we have very little knowledge of what the evocator does to the ectoderm when it reaches it. The concept of a chemical stimulus, whether specific or not, is clearly very inadequate in this connection. If we are dealing with a simple, fairly rapid response, such as that of a nerve, the notion of a stimulus may be appropriate; but in discussing evocation we are really attempting to determine the conditions under which a complex mixture sets out on one, rather than another, course of

reactions; and these reactions themselves are by no means simple, but involve a co-ordinated set of syntheses of different cell proteins, as well as the histological arrangement of these substances into characteristic patterns. No chemist, I think, would discuss organic syntheses in terms of stimuli, and such a formulation seems merely to obscure the necessity to find out what the conditions are under which the different syntheses take place, and what are actually the crucial effects of the so-called stimulating substances.

We have seen that in practice several different classes of chemical compounds can bring about evocation. The mere elaboration of the hypothesis that evocation is none the less a response to a specific stimulus, or to one of a small group of stimuli, does not enable us to decide which of these compounds is the specific evocator; no amount of hypothesis can make the experimental data more definite than they are. But with the adoption of this hypothesis, the question of the identification of the evocator becomes meaningful and important, and requires discussion.

The compounds which come into question are the sterol fraction of tissue extracts, the carcinogenic and oestrogenic hydrocarbons, the fatty acids and nucleoproteins used by Fischer and the cephalin and digitonin used by Barth. When one examines the concentrations at which these substances have been employed, it is clear that there are very large differences between them. The sterol fraction, for instance, is active in concentrations as low as 0.1 mg. per c.c. which corresponds to a dose of about 0.06 mg. per kg. wet weight. Shen¹ has made a very exact study of the activity of the water-soluble sodium endosuccinate salt of 1:2:5:6-dibenzanthracene, and finds a maximum of activity at a dose corresponding to 0.25 mg. per kg.; there was noticeable activity, however, at considerably lower doses (Fig. 4). The activity of most substances of other types is much lower; the fatty acids and cephalin are used at concentrations of about 5 per cent which corresponds² to a dose of 3150 mg. per kg., while the nucleoproteins were used undiluted, giving a dose of about 62.5×10^3 mg. per kg. Only the digitonin is of comparable activity, being active at 0.05 per cent, or at a dosage of 31.5 mg. per kg.³

¹ Shen, 1939.

² The following figures were given incorrectly in Shen's paper.

³ Barth, 1937; Barth & Graff, 1939.

Even at this dose, which is somewhat larger than is necessary for substances of the steroid type, the activity was very weak, the "inductions" consisting merely of feeble palisades, whose neural nature does not seem beyond question. Even if we accept the activity of digitonin in such concentrations, however, the fact

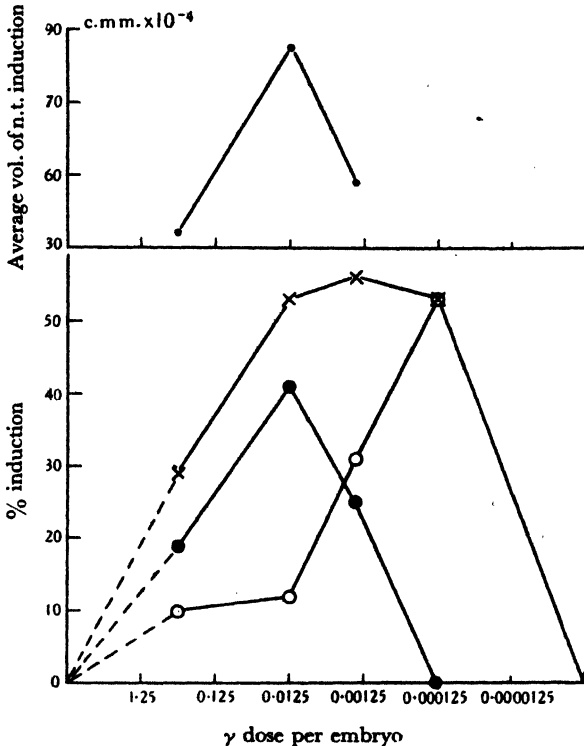


Fig. 4. Percentage of inductions, and volume of neural tube induced, at different doses (in mgm. $\times 10^{-3}$ per embryo) of 1:2:5:6-dibenzanthracene sodium endosuccinate. \times all inductions, \bullet neural tubes, \circ palisades. (From Shen.)

that it is a well-known cytolyzing agent certainly suggests that it acts in an indirect way, by causing the ectoderm to release its own evocator.

We are left with the conclusion that it is the steroid substances which are the most active evocators, and we may therefore advance the tentative hypothesis that they act "directly", being perhaps as closely related to the natural evocator as they are to

the natural sex hormones. It is interesting to note that the optimal dosage for the steroid evocators mentioned above is of the same order of magnitude as that of the natural sex hormones.

The substances which have been referred to as steroids form a remarkable group of biologically active substances which includes the sterols, the oestrogens, carcinogens, cardiac poisons, vitamin D, colchicine, and many alkaloids. The different activities of these compounds are probably related in some as yet unknown way, since not only can several members of the group produce the same stimulus, but the same member can also produce different stimuli. Thus several oestrogenic substances are known, and some compounds are both oestrogens and carcinogens. Needham¹ has symbolised the relations in a diagram in which the different activities, such as carcinogenesis, oestrogenesis, etc., are pictured as circles overlapping in a field on which the various compounds are scattered.

Evocation can certainly be included among these overlapping activities. Tests have recently been made of quite a number of these compounds, and both in the newt² and the chick³ evocation was obtained with several.² Strong evocatory activity was only shown by compounds known to be active in other biological reactions. The strongest reaction from an otherwise inactive substance was from naphthalene, but the preparation was not highly purified, and the apparent activity may well have been due to an impurity. Some weak evocatory power, however, was found in some substances whose other biological activity is negligible, such as anthracene. It is noteworthy, also, that the water-soluble sodium endosuccinate of dibenzanthracene showed considerably greater activity than the water-insoluble dibenzanthracene itself which had been tested previously. Making all due allowance for possible differences in the two sets of eggs employed in the different tests, this difference in activity is almost certainly real. It suggests that the difficulty of diffusion of a fat-soluble substance from the emulsions employed may be an important factor in the tests.

The mode of action of the members of the "steroid" group of substances is unknown. Some of them, such as the naturally occurring oestrogens, presumably have a "direct" influence on the chemical processes proceeding in the reacting tissue such as

¹ Needham, 1936.

² Waddington, 1938*a*.

³ Abercrombie, 1939.

the vaginal mucosa; at least, this can be taken as a definition of what is meant by direct in this context. Other oestrogens may, perhaps, be converted into oestrins before acting. Similarly, the carcinogens may have a direct action, or may be converted in the body into something else which has a direct action, or finally they may release substances which are present but inactive in the tissues. Such questions cannot be answered until we know something of the nature of the chemical changes involved in the tissue reactions which occur in oestrogenesis or carcinogenesis. As regards the evocator, the hypothesis of a specific stimulus, which we are discussing, would suggest that the normal evocator acts "directly" in the same sense that normal oestrogens do, while other "steroid" evocators have the same relation to the normal evocator as the steroid oestrogens have to the normal oestrins.

Two further facts about the evocator require mention, though neither of them allows us to draw any definite conclusions as to its chemical nature. In the first place, Holtfreter¹ has shown that evocation may be obtained with very many different animal tissues, which may come from any part of the animal kingdom from mammals to coelenterates. He failed to obtain any inductions with plant material, and later claims that plant material is active would seem, from the published evidence, to be open to some doubt.² If all these inductions are due to the normal evocator, that must be a very widely distributed substance. However, there is no reason to suppose that the normal evocator is always concerned. Most of the tissues were killed before implantation, and many of the remainder must have suffered some degenerative changes during the period immediately after implantation. They may therefore have released substances, such as acids, which act as secondary evocators.

Holtfreter² also showed that it is extremely difficult to deprive tissue of evocator activity by extracting it either with water or with fat solvents. This might be due to an intimate relation between the fat-soluble evocator and some other substance such as glycogen or protein; or it may be due to the liberation, during the extraction, of compounds capable of acting as evocator liberators. No definite conclusions can be drawn where so many possibilities are open. Certainly one cannot conclude, from the

¹ Holtfreter, 1934 *b*.

² Holtfreter, 1934 *a*.

Ragosina, 1937; Toivonen, 1938 *a*.

fact that tissue extracted with a certain solvent does not lose its power of evocation, that the normal evocator is not soluble in that solvent.

The inductions which have been reported by proteins require separate discussion. It has been known since the beginning of the chemical work that inductions may be obtained with somewhat crude protein preparations, such as the watery layer in the centrifugate of embryonic material, and also the residues left after ether extraction of tissues. The possible presence of traces of ether-soluble materials in such preparations could, however, not be neglected, and it was not clear that the inductions were due to the proteins themselves. Barth¹ has obtained further inductions with relatively impure protein extracts, and has claimed that bigger and better inductions are obtained with protein residues than with ether extracts, but this is at least doubtful, especially when the effect of diffusibility is eliminated by comparing the protein extracts with water-soluble substances related to the ether-soluble hydrocarbons, such as the sodium endosuccinate of dibenzanthracene.

The possibility that proteins may themselves act as evocators has, however, become much more immediate through two recent discoveries. One is that certain preparations of egg albumen may be strongly evocating, even after recrystallisation, although other preparations are equally certainly negative.² The second set of data have been discovered by Brachet and Rapkine, who state that certain proteins, inactive in the native state, can become evocators when their $-SH$ groups are unmasked, and further claim to be able to suppress the development of ectoderm into neural tissue, even in the presence of an organiser, by controlling its oxidation-reduction state.³ It is still too early to discuss this work in detail, since all the data do not yet appear to be consistent; for instance, there is no doubt that some preparations of albumen are inactive even after boiling, which should liberate all the sulphhydryl. But the line of work seems one of the most likely which has yet been started to lead us to a knowledge of what actually occurs during evocation. It seems unlikely that protein sulphhydryl can act indirectly as an unmasker; on the contrary, it is much more likely to be itself a product of an unmasking. It will be extremely

¹ Barth, 1939; Barth & Graff, 1938.

² Shen (unpubl.)

³ Brachet & Rapkine, 1939.

interesting to learn more of the relation between it and the other evocators which apparently act directly, namely the steroid substances, and this is one of the foremost tasks in the investigation of chemical evocation to-day.

The Activation of the Evocator.

The hypothesis that some of the evocating substances act secondarily, by first liberating the natural evocator, was originally suggested by considering the ease with which non-evocating presumptive ectoderm can be converted into organiser. A natural extension of the same line of thought is to ask whether some similar process occurs in normal development. Is there some mechanism located in the region of the blastopore, which liberates the evocator in the tissue there?

The first result of such a query was a long, but successful, shot in the dark. The insistence by Child and many subsequent authors on the importance of respiratory metabolism for morphogenetic events suggested the guess that peculiarities of the respiratory metabolism might be the release mechanism. This suggestion was no doubt vague; the phenomenon under consideration is not by any means exactly analogous to those dealt with by Child's theory. But the guess was taken seriously enough to suggest exposing presumptive ectoderm to respiratory catalysts, and it was for this reason that the experiment of cultivating such tissues in methylene blue was first undertaken. The discovery that evocation resulted was, as has been said, a final proof that evocation can be produced by many different compounds. Thus the hypothesis of evocator activation which has been discussed in the previous section was not developed after a series of inconvenient facts had been discovered, but actually preceded and predicted the discovery of these facts.

The mere production of evocation by methylene blue, encouraging though it was, did not of course suffice to clinch the whole line of argument. It is, in the first place, necessary to show that there actually is a peculiar respiratory metabolism in the organiser region, and that the action of methylene blue on the presumptive ectoderm is such as to make it assume this peculiarity. Investigation of these two points has already progressed some distance, though they are neither of them fully cleared up.

The first point of attack was on the oxygen uptake and CO_2 output of the organiser and of comparable material from the

ventral side of the egg. Brachet¹ was able to show that the CO₂ output is considerably higher (in the ratio 1.89:1.00) in the blastopore lip than in equivalent ventral material. Measurements of oxygen uptake at first led to some contradiction. Brachet originally found a somewhat higher uptake in the organiser region. Repetition of the measurements with a more sensitive manometer gave results which did not differ significantly between the dorsal and ventral sides.² In still other measurements, Brachet and Shapiro³ compared the oxygen uptake of the dorsal and ventral sides of uninjured gastrulae, and found a considerable excess for the dorsal side; but as the invagination proceeds and the ectoderm becomes underlain by mesoderm, the dorsal side becomes double the thickness of the ventral, so that the significance of its higher oxygen uptake is doubtful. Fischer and Hartwig⁴ found a small excess (about 20 per cent) in the organiser region, but the accuracy of the instruments which had been used up to that time made a difference of this magnitude hardly sufficient to be accepted with confidence. The most recent measurements are those of Needham and his collaborators,⁵ made with a modification of the Cartesian diver manometer which is very much more sensitive than any of the earlier instruments. They find no significant difference in oxygen uptake between organiser and ventral ectoderm. This probably reflects the true state of affairs; at best the difference between the two tissues cannot be large.

The adaptation of the Cartesian diver to measurements of embryonic metabolism is perhaps one of the most important technical advances which have been made in experimental embryology, since it allows the biochemical investigation of quite small regions of a single gastrula. Needham and his co-workers have already been able to measure the intensities of several metabolic processes which previously were quite out of reach. These intensities have been related to the nitrogen content of the metabolising fragments, since this seems the best available method of measuring the quantity of metabolising material; even this, however, is not entirely satisfactory, since the cells are full of yolk, which presumably takes little part in respiratory metabolism, but yet contains nitrogen. However, the nitrogen content is probably

¹ Brachet, 1934*b*, 1939.

² Waddington, Needham & Brachet, 1936*a*.

³ Brachet & Shapiro, 1937.

⁴ Fischer & Hartwig, 1938.

⁵ Boell, Needham, Rogers & Koch, 1939.

a somewhat better index than the dry weight, and no better measure has yet been suggested.

The most interesting, and the most definite, information about the metabolism of the organiser concerns the glycolysis. Woerdemann¹ originally showed by histochemical methods that there is a rapid disappearance of glycogen in the invaginated mesoderm, and this was confirmed by microchemical estimations by Heatley and Lindahl.² The Cartesian diver measurements have shown that the anaerobic glycolysis of the organiser region is about three times as high as that of the ventral ectoderm; and it seems clear that this must be connected with the disappearance of the glycogen. Moreover, it was known from experiments of Brachet³ that the respiratory quotient of the whole egg is about 0.7 at the beginning of gastrulation and rises to nearly 1.0 at the end. The diver measurements indicate that this change occurs earliest in the organiser region and spreads from there over the rest of the gastrula. Less specific evidences of metabolic peculiarities in the organiser region were found in the older experiments of Bellamy and Bellamy and Child,⁴ who showed that various injurious agents had a more rapid action on this region. Similarly, Fischer and Hartwig, and Piepho,⁵ studied the reduction of vital dyes and Piepho found a more intense oxido-reductive metabolism, which might be either respiration or glycolysis, in the organiser. Rulon,⁶ with similar methods, finds a more intensive reduction in the primitive streak region of the chick. The reduction of dyes also provides evidence that there is a gradient of oxido-reductive activity from the animal to the vegetative pole of the amphibian egg; and Heatley and Lindahl described a similar gradient in the glycogen content. The metabolism of the animal pole has not yet been investigated manometrically, though this is much to be desired.

Finally, Brachet⁷ has shown, by histochemical methods, that there is in the organiser region a marked differential concentration of proteins giving rise to sulphhydryl groups on denaturation. Substances possessing this property are almost confined to the germinal vesicle in the unfertilised egg, but soon after fertilisation are found in the animal pole region, and, before the occurrence

¹ Woerdemann, 1933.

² Brachet, 1934*a*.

³ Fischer & Hartwig, 1936; Piepho, 1936.

⁴ Rulon, 1935.

⁵ Heatley & Lindahl, 1936.

⁶ Bellamy, 1919; Bellamy & Child, 1924.

⁷ Brachet, 1938.

of the first cleavage, accumulate in the future organiser region. Brachet attributes a very important role in induction to such substances, apparently considering that they may act directly as evocators. It is certainly remarkable that, according to Rulon, sulphhydryl groups are also concentrated in the primitive streak in the chick, so that there are some grounds for supposing that their association with induction is general and important.

Jacobson,¹ studying the histochemistry of gastrulation in the chick, was able to show that in that form also there is a very rapid disappearance of glycogen, both during the invagination of the endoderm and of the mesoderm. He found also a particularly high concentration of lipoids, which seemed to be sterols, in the primitive streak, and suggested that this might be due to a liberation of these substances in the way we are discussing.

These investigations make it quite clear that the organiser region has, at least from the beginning of gastrulation onwards, a metabolism different to that of the rest of the egg. The hypothesis that the evocatory power of the organiser is due to the liberation within it of the evocator substance therefore survives its first test; at least there are metabolic peculiarities which could cause a liberation. Moreover Brachet² claims to have evidence that the metabolic peculiarities are actually linked to the evocatory power, since both are suppressed by treatment with monoiodoacetate.

The second question raised above was whether methylene blue actually changes the metabolism of ectoderm into that characteristic of the organiser. Here the evidence is as yet meagre and indecisive. Beatty, de Jong and Zielinski³ found only a slight elevation of oxygen uptake in pieces of ectoderm submitted to methylene blue, but we have seen reason to doubt whether it is the oxygen uptake which is the important characteristic of the organiser, and the effect of dyes on the anaerobic glycolysis has not yet been tested. On the other hand, some doubt is cast on the role of methylene blue by the same author's report that evocation can be obtained with other dyes, which include not only other respiratory catalyts such as pyocyanin but also neutral red and Janus green, which have a considerably lower rH . The inductions obtained, however, were very slight and further work on these lines is required.

¹ Jacobson, 1938.

² Brachet, 1934 b.

³ Beatty, de Jong & Zielinski, 1939.

The liberation hypothesis, however, does not stand or fall by the particular mode of liberation which methylene blue produces. Waddington, Needham and Brachet, in the paper in which the hypothesis was first put forward, drew attention to the possibility that methylene blue may act in some other way; for instance, it has been reported to produce a slight denaturation of protein, and it is possible that this is the cause of the liberation of the evocator.

Finally, we may enquire whether the data allow of any suggestions as to the reaction involved in the evocator liberation. Spemann, Fischer and Wehmeier¹ originally suggested the presence of an anti-evocator which was destroyed by such processes as boiling, drying, etc. It was not easy to picture a substance with the properties actually required to ensure its disappearance under all the appropriate conditions, and the hypothesis has not been elaborated further. As an alternative, Waddington, Needham and Brachet suggested the idea that the evocator may, before liberation, be linked in loose chemical or physical combination with some other substance, and that the liberation consists merely in the loosening of the bond. Such a loosening may be produced in a great number of ways, and the ease with which the evocatory power is awakened may be easily pictured. Among candidates for the evocator's partner, glycogen comes first to mind, since it is obviously involved in the metabolism of the organiser, and crude glycogen preparations have been shown to be capable of evocation. No difference in evocatory power was found between glycogen prepared from that fraction combined with protein in the cell (desmo-glycogen) and glycogen in the readily extractable form, but this is not really the point at issue.² It is quite possible, however, that protein may also be included in the evocator complex before liberation; this is suggested by the fact that the evocator is set free by any coagulation of the cell proteins.

A final question which needs discussion is the time at which the liberation of the evocator can be supposed to occur. Most of the metabolic investigations have been concerned with early gastrulation stages. Thus the diver and manometer experiments were performed on tissue just before it became invaginated; while the analyses of glycogen content were made in tissue after invagination.

¹ Spemann, Fischer & Wehmeier, 1933.

² Heatley, Waddington & Needham, 1937.

The disappearance of glycogen, in fact, does not occur until gastrulation nor does the change in R.Q., but there is no direct evidence of when the other metabolic differences are first initiated. They may be present much earlier than they have yet been discovered. Certainly Brachet has shown that the concentration of proteins giving rise to free sulphhydryl in the organiser region occurs very early, in fact soon after fertilisation. Attacking the problem from the other side, we have some evidence that the evocator is actually free in the organiser region some time before gastrulation. Thus Mayer,¹ who brought gastrula ectoderm into contact with the presumptive organiser region of early cleavage stages, has reported that inductions were produced.

Whatever the time at which the evocator is normally liberated, the hypothesis of secondary action which we have advanced here requires only that the liberation shall still be possible as late as the gastrula stage. The evidence for this has been discussed above; and, as far as I know, there is no evidence against it.

¹ Mayer, 1939.

CHAPTER V

COMPETENCE

THE notion of competence implies an ability to react; it is similar to the idea referred to in German as *Reaktionsfähigkeit*. It is tested by finding out what types of development a piece of tissue can be caused to carry out when submitted to various stimuli. A piece of tissue is competent to form a certain organ or tissue during the period in which it becomes determined either to form that organ or not to form it.

It is important to compare the idea of competence with that of potency. A potency implies an ability to perform an action. In embryology it is used to describe an ability to develop. It is essentially tested by reference to a future condition; we can say that a piece of tissue has a potency to develop into something if, in the course of time, it does become that thing. Such a reference to a future happening is of course a necessity in all experiments concerned with development. But it is of the utmost importance to deduce, from future happenings, something about the present conditions within the material investigated. The idea of potency does not involve any valid deductions of this kind; it does no more than reflect the future happenings directly back into the material. To say that a fragment of tissue has a potency to develop into *A* says no more about the fragment than that it can develop into *A*. In contrast with this, the notion of competence definitely implies something about the present condition of the tissue, before it performs its development. The competence for *A* is a reactivity, that is to say, it is a state which characterises the tissue at the time when the experiment is performed from which the presence of the competence is deduced.

The idea of potency, then, is a purely formal one. It does not provide a working hypothesis, in the sense of attributing to the potent tissue any immediate properties which can be investigated. Thus a theory like that of the segregation of potencies,¹ so much favoured by American authors, seems to be merely verbalistic. The potencies are not real objects which can be "segregated";

¹ Lillie, 1929.

they can be tested only by future development, and in fact are no more than future developments, verbally transferred to an earlier stage.

On the other hand, we have seen that when a tissue is competent, it can carry out certain reactions which it cannot perform at any other time, namely reactions with the appropriate organiser. It is true that the test of the occurrence of this reaction is the performance of a certain kind of development in the future, but that does not alter the fact that the reaction takes place at the exact time when the competence is said to be present. There is no question here of a mere verbal transference of events from one time to another. The presence of a competence tells us something about the actual state of the tissue in question, and, by investigating the properties of competences, we can frame certain hypotheses as to the material basis of such states.

The Origin of Competence.

Competence has been defined above by reference to the process of determination. This has the practical disadvantage that it is difficult to discover when a process of determination begins. But this disadvantage cannot be sidetracked without rendering the notion of competence altogether abstract and unconnected with the chemical processes which we eventually hope to discover. Let us suppose, for example, that the process of determination of the neural plate begins when the ectoderm is first underlain by the mesoderm; now let us perform the experiment of placing an isolated fragment of blastula ectoderm in contact with an evocating substance and removing it again before the time at which the mesoderm would normally reach it. It is actually found that such a fragment of ectoderm develops into neural tissue.¹ Shall we say, then, that it was competent during the period of application of the evocator? If we wish to think in terms of the chemical processes which cause determination, the tissue should only be considered competent during the time when the evocator process is actually occurring; the mere time at which the evocator diffuses into the cell is not so important as the time at which it begins acting. If we assume that the determination process cannot begin until the time the mesoderm normally reaches the tissue, the ectoderm in the above experiment should not be considered to

¹ Waddington & Beatty (unpubl.).

be competent during the period it was in contact with the evocator. But, actually, there is no experimental evidence which determines when the evocator reaction first begins, and when the notion of competence therefore first becomes relevant. The only hint we have on this point is that induced neural tissue never seems to appear much earlier than the neural tissue of a normally developing host embryo, either in Amphibia or birds, so that it appears likely that the evocation reaction cannot begin much earlier than it actually does in normal development.¹

During development, the embryonic tissues pass through a succession of competent phases. Thus the ectoderm reacts first to the primary organiser by the formation of a neural plate, then to the eye-cup by the formation of a lens and so on. Even though we cannot identify exactly the times at which the different competencies first occur, we should be able to study the details of this succession. Actually, rather little work has been performed on this problem. One question which has been raised is whether the occurrence of a later competence is dependent on the previous interaction of the tissue with an earlier organiser. The first instance in which this was investigated was in the chick.² Here the embryonic endoderm induces a primitive streak in the epiblast, and later the remainder of the epiblast becomes competent to form neural tissue under the stimulus of the primitive streak organiser. In the extra-embryonic regions, the embryonic endoderm is lacking, and it is interesting to enquire whether the endoderm here acquires the neural competence. It was found that grafts of the primitive streak in the *area opaca* gave clear neural inductions, whence one must conclude that the development of this competence is independent of the endoderm organiser.

Similarly, ectoderm of the young amphibian gastrula may be removed before it has been acted on by the primary organiser, cultivated for some time *in vitro* and then submitted to the action of a later organiser such as the eye-cup. It is found to have developed the appropriate competence and to form an induced lens.³

These results give the impression that the development of competence is completely autonomous and that a given piece of

¹ But see Filatov, 1937.

² Waddington, 1934*a*.

³ Waddington, 1936*b*; Filatov, 1937; Holtfreter, 1938*b*. Cf. also Lopaschov, 1935*a*.

tissue inevitably passes through a definite series of competent states whether or not it has been submitted to the normal series of inducing stimuli. This, however, cannot be generally true. In the first place, in all the experiments which have yet been made, the normal series of inducing stimuli has only been interrupted for a short period. Lens competence arises very soon after the neural competence of the gastrula, and the fact that its origin is independent of the neural organiser cannot be taken to indicate that much later competences could also be formed in isolated gastrula ectoderm. Moreover, even the origin of lens competence is not entirely automatic. When fragments of gastrula ectoderm are isolated, they tend to roll up into small balls, which may remain hollow or may collapse into solid lumps. Lenses were induced only in the former, and it seems that lens competence can only be exhibited by comparatively thin layers of ectoderm. Finally, the competences whose origin has been investigated in this way are those of the epidermal derivatives, and there seems no reason to doubt that in normal development the neural organiser has a very much less important action on the epidermis than on the neural plate; in fact, it is a matter for discussion whether it has any definite action at all on the former. Again, therefore, we should be making an unjustifiable extrapolation if we deduced, from the cases which have been described, that all competences arose autonomously.

In some cases, in fact, it is clearly impossible to invoke such an autonomous origin. For instance, the formation of lens fibres may be induced by a stimulus from several regions of the neural system, but they can be formed only in lens tissue, and the lens-fibre competence cannot be independent of the previous induction of the lens. Similarly, the eyes are induced by the anterior end of the roof of the primitive gut, but this induction cannot occur in tissue which has not already been induced to be neural plate. In general, in all cases in which competence only arises in a limited region, we must suppose that it is dependent on the completion of a definite sequence of developmental events.

The Nature of Competence.

The conditions in these different cases may appear somewhat paradoxical. But some of the difficulties disappear if we form a picture of what competence may mean. In the first place, it is a state of instability, since it involves a readiness either to react to an

organiser and follow a certain developmental path, or not to react and to develop in some other way. Moreover, this instability occurs in a system which, both before and after the competent period, is in process of developmental change. This change must be brought about by the interaction of chemical substances, and physical conditions, within the tissue. The whole process of development may therefore be considered as resulting from an unstable configuration of substances which leads the embryonic tissue to change towards a more stable state; and the periods of competence are secondary instabilities when there are two or more alternative modes of progression towards stability, the choice between them depending on outside conditions (the presence or absence of the appropriate organiser). One can compare a piece of developing tissue to a ball running down a system of valleys which branches downwards, like a delta; I have also used an analogy with a shunting yard in which the wagons are moved by gravity. The tissue, like the ball or the railway wagon, must move downhill, but at some points there are two downhill paths open to it. At such branching points, it may sometimes require a definite external stimulus, such as an evocator substance, to push the tissue in to one of the developmental paths; in such a case, competences which occur later along this path will only be developed if the evocator has acted. In other cases, a certain path may be followed merely because an evocator has failed to be present, and then the subsequent competences may appear to develop autonomously; this is probably the case with the epidermal competences we considered earlier. Sometimes the "positive" action is not on the side one would expect. Thus most organs are only formed in response to a definite inducing stimulus, but in anurans the ectoderm has an inherent tendency to form suckers, which has to be restrained in the non-sucker region by an inductive influence of the mesoderm.¹ This might be called a negative induction.

This idea of competence is clearly allied to Lillie's² notion of "differential dichotomy" or segregation, which also involved the suggestion that at certain periods of development a decision has to be taken between alternative modes of change. Lillie's scheme, however, was defined simultaneously with respect to two different, and in some cases incompatible, processes. According to Lillie, segregation is the "process of origin of parts of the embryo

¹ Yamada, 1938b.

² Lillie, 1929.

possessing irreversible prospective value as tested by self-differentiation". I have already pointed¹ out that self-differentiation is no test of irreversible prospective value, and at the same time I discussed some of the objections to the manner in which the concept has actually been employed in the literature.

The most valuable feature of Lillie's idea was that it did not consider only the restriction of potencies during development, but also indicated that new capacities arise in tissues during ontogeny. In this respect it comes very close to the idea of competence. Spemann² has also insisted on the necessity of recognising that development involves not only restrictions but also the acquisition of new capacities, and he quotes an eloquent passage from Vogt³ to this effect. Both these authors, however, include this increase in capacity within the general phenomenon of determination. We have already pointed out that Spemann sanctions a use of the concept of determination which is so wide as to deprive it of all causal value. I have suggested that we take a more precise point of view and define determination with reference to a particular process of development rather than to the production of a particular organ. Here I am suggesting a still further analysis of such processes, by considering them under the heads of the arising of a competence to react, and of the carrying out of the reaction. Only the latter of these two is determination in the sense in which that word is used in this book.

The notion of competence, like that of determination, should be referred to a process rather than to an end-product. For instance, lenses may be induced from several different embryonic tissues, including neural tissue and mesenchyme as well as epidermis;⁴ they can be formed, in Wolffian regeneration, from the iris; and Schotte⁵ has recently shown that even a regeneration blastema from the tail can be caused to develop into a lens. All these tissues may loosely be said to possess lens competence. But further work is required before we can say whether the lens competence in all cases means the same thing. Presumably differentiation into a lens involves the synthesis of specific lens proteins whose properties cause the formation of a small vesicular mass, and which later become capable of forming the lens fibres. It may be that the processes of synthesis are the same, whether we start with

¹ Waddington, 1935*a*.

² Spemann, 1938.

³ Vogt, 1927.

⁴ Popoff, 1937.

⁵ Schotte & Hummel, 1939.

embryonic epidermis, neural tissue, mesenchyme or regenerating cells from an adult. If so, the lens competence is really always the same thing. But it is quite possible that the substrates and raw materials are different in the different cases, when one would have to conclude that the competences were only superficially similar in so far as they produce similar end-products.

The idea of competence which has been advanced here does not necessarily imply a dichotomy; that is to say, during a period of competence there may be more than two alternatives open to a tissue. Cases are known where this is undoubtedly the case. Gastrula ectoderm, both in Amphibia and in birds,¹ can develop into mesoderm as well as into neural plate or epidermis, and has therefore at least three alternatives open to it.

We must also consider the possibility that the alternatives are not sharp; there may be intermediate types of development which are possible. In connection with the neural organiser we have discussed the neuralised thickenings, which can perhaps be regarded as intermediate between epidermal and truly neural development. The importance of such intermediate types of differentiation will depend on how far the reaction of competent tissue to the evocator is purely quantitative and how far threshold phenomena are involved. In general, we tend to find that in embryonic development the alternatives open to competent tissues are fairly sharply contrasted and that intermediate types of differentiation are correspondingly rare. This is clearly necessary if the mechanism is to be efficient and reliable, since the function of the mechanism is to produce the sharply specialised tissues of the adult. There must, therefore, be a strong pressure of selection during evolution tending to cause the formation of developmental systems which have only certain well-defined types of change open to them. These developmental potentialities will be dependent on the genotype, and we shall see that a consideration of the effects of genes on development, as far as they are yet known, leads to the formulation of a similar scheme of sharply alternative types of differentiation.

The Loss of Competence.

The disappearance of competence can be investigated considerably more easily than its origin. If organisers of a given type are implanted into embryos of different ages, it is found that there

¹ Waddington & Taylor, 1937.

is a gradual falling off of competence with age, which may be exhibited either as a reduction in the frequency with which an induction occurs or as a diminution in the average size of the inductions or in both ways. In both the chick and the newt, the competence to form neural plate disappears only shortly before the first signs of neural-plate formation can be seen. Peculiarly enough, it vanishes earliest in those regions (the posterior) in which the neural plate appears latest.¹ In other cases there may be a longer interval of time between the end of the competent period and the appearance of the organ, while in still other cases, the competent period may persist after part of the tissue has already begun to differentiate into the organ in question (cf. the regeneration of lenses from ectoderm during the tail-bud stage in newts).

The disappearance of competence, like its origin, may sometimes be autonomous. For instance, if gastrula ectoderm of the newt is isolated in salt solution, it eventually loses its neural competence.² This loss takes place gradually, and the end of the period of neural competence, when only neuralised thickenings can be formed, may overlap the period of lens competence. The loss occurs more slowly than in intact eggs. It was found that ectoderm, removed from the young gastrula and cultivated until its age corresponded to the open neural-plate stage, could still show some neural response, although in the intact egg this response would have entirely vanished. One might attribute this slowness to the absence of some action normally exerted by the organiser on the epidermis, but it is perhaps equally plausible to suggest that in normal development the loss of competence is hastened by the stretching of the epidermis to a thin layer covering the inner organs of the embryo.

Loss of competence seems frequently to proceed by a spatial contraction of the competent region. Thus in *Rana esculenta* the entire epidermis is lens-competent in the early neural stage; a little later only the epidermis of the head can react; and later still only the presumptive lens epidermis. The boundary of the competent region is of course not sharp; the whole phenomenon is one of a quantitative variation.³ As the peripheral parts of the epidermis lose their competence, the presumptive lens and a small region near it acquire a capacity for self-differentiation. This would

¹ Machemer, 1932; Woodside, 1937.

² Waddington, 1936*b*; Holtfreter, 1938*b*.

³ Cf. Spemann, 1938.

be a rational consequence of the gradual resolution of a state of instability in which two alternatives were present; in the peripheral regions the constituents of the cells are altered in such a way that one of the two alternatives is adopted, while the central regions adopt the other. Thus as it were reciprocal to the loss of competence is an increase in the capacity to complete one or other of the two types of differentiation independently of any further external stimulus.

The most remarkable fact which has been discovered in this connection is the occurrence of what is known as *Doppelte Sicherung* or double assurance. In *Rana esculenta* the eye-cup can induce lenses in non-presumptive lens ectoderm, and therefore presumably can affect the ectoderm which normally forms the lens; but if the eye-cup is removed, a lens nevertheless forms, so that the inductive action of the eye-cup is unnecessary. If this is thought of simply in terms of the capacity for self-differentiation or the necessity of an inductive stimulus, it seems a somewhat mysterious and complicated arrangement. But if the reaction of competent tissue always consists in the resolution of a state of instability in one of two or more possible ways, it is only to be expected that the decision can sometimes be produced by an action much less prolonged than that which the organiser actually provides in normal development, so that the tissue becomes self-differentiating at a time when the organiser is still active.

In fact, one would expect that in general the more highly developed the competence, that is to say the more sharply the alternatives are contrasted, the smaller the external stimulus which will be necessary to decide between them. The evolution of a really efficient competence may therefore be expected to reduce the importance of the evocator, which will probably tend to disappear; and we may expect to find cases in which the functions of the evocator are so slight that they are taken over by minor variations in conditions which are very difficult to identify. Phenomena of this kind may lead to the evolution of a mosaic type of development from a regulative; and they may also help to explain cases, such as that of the callosities of the ostrich, in which structures which are apparently adaptive responses to external stimuli actually develop before the stimuli can possibly be present.

It was one of the merits of Lillie's hypothesis of differential dichotomy that it was supposed to apply to mosaic development

as well as to regulative. We have here reached the same type of conclusion from a slightly different point of view. The evocator-competence reaction is found when diffusion of an active substance is an essential part of the developmental mechanism. But the actual processes of differentiation can be explained only in terms of the reaction to this stimulus. The transition from typical inductive development to cases of double assurance show that the processes occurring in mosaic development, where no organiser is involved, are of the same general nature as those involved in competence; only in the former the factor which decides which mode of development will be followed by a given piece of tissue is already present within it, instead of having to diffuse in from the surroundings.

An alternative way of regarding this is to consider the localisation of the evocator within the egg at the time of fertilisation. In the amphibian egg, the neural evocator, or some precursor condition, is localised in the grey crescent region, where it is associated with other conditions which cause this area to differentiate into mesoderm, while the presumptive neural-plate material lies farther towards the animal pole. One has only to imagine that the neural evocator was from the beginning localised in this more animal region to have a picture of an egg which would show the typical phenomena of mosaic development and organ-forming substances. Clearly, by altering the localisation of a single substance, one would not make any very fundamental change in the whole complex of reactions which must be involved in the differentiation of the nervous system. All the rest of this complex, which is by far the greater part of it, can be imagined as exactly the same in the mosaic as in the regulative type of development.

The Evocator-Competence Reaction.

It is still necessary to discuss the question of whether the evocation of competent tissue produces an organ or a tissue. The neural evocator cannot of course by definition produce a specific organ of the nervous system, but that does not necessarily imply that it must produce only a chaotic mass of tissue; it might produce organs at random, sometimes a brain, sometimes an eye, sometimes a trunk, etc.

The available evidence on this point is conflicting and is as follows. Implantations into whole eggs are not very suitable for

testing the reactions to evocators in this respect, since the influence of the host may not be negligible. In implants into isolated flaps of ectoderm, I have very rarely observed anything like organ formation, either when chemical substances have been used or when dead parts of the amphibian egg have been employed. Very large neural inductions may be obtained which show no sign of any organ differentiation, unless one can regard some rather ambiguous ectodermal thickenings as signs of the formation of nasal placodes. In one or two cases, however, fairly definite eyes, with associated lenses, are present, and these have been found also with chemical implants. Chuang,¹ using implants of liver and kidney, found considerably more organ formation. With liver, which evocated only neural tissue, the only organs which could be recognised were derivatives of the brain, but kidney, which induced mesodermal tissues as well, caused the appearance of well-formed tails. The evidence, I think, suggests that the tissues are at first disorderly, and often remain so, but that they have a tendency to become arranged, in a more or less haphazard way, into organs. It is noticeable that the eyes which occurred in Chuang's and my experiments were often imbedded in a mass of chaotic neural tissue; they were not regularly attached to a brain, in the normal way. It is probable that when two or more tissues are induced, such as mesoderm as well as neural tissue, the mutual arrangement of the tissues to form organs proceeds more readily and comprehensively and thus gives rise to the well-developed tails by Chuang.

Shen (unpublished) has recently studied the matter in what is perhaps the most critical case—with a single pure active substance (the sodium endo-succinate salt of 1:2:5:6-dibenzanthracene) dissolved in the salt solution in which the explants were kept. He found no sign of organ formation, although the large induced neural masses in some cases had a closer resemblance to brain tissue than to neural tube. Quite often there were indications of radial symmetry, which is what one might expect since the evocator was applied simultaneously from all sides.

The Genetic Control of the Evocator-Competence Reaction.

We have seen that the production of new types of tissue during development is normally, in vertebrates at least, by means of a reaction between an evocator and competent cells. The evocator

¹ Chuang, 1938.

is a chemical substance, and we have been led to suggest that competence is a state of instability in a system of interacting substances. In other eggs with a mosaic type of development, the essential features of the developmental system are probably not very dissimilar.

The ultimate source of these substances must be the constituents of the fertilised egg; cytoplasm and nucleus. There should at this time be no need to argue the point that, from the evolutionary point of view, the nucleus, and in particular the genes, is the more important of these. It is of course true that neither nucleus nor cytoplasm can long support life when dissociated from the other. Of the two, probably the enucleated cytoplasm can persist longer than the naked nucleus, and it can even carry out some biological activities, such as the cleavage of enucleated cells described by Fankhauser and Harvey.¹ But it is well known that many substances which are certainly under genetic control are, by the time we examine them, located not in the nucleus but in the cytoplasm; examples are pigments, immune bodies, etc. The responsibility for some, at least, of the properties of the cytoplasm must therefore be attributed to the nucleus, and the mere fact that the cytoplasm of a given cell can perform certain actions does not necessarily mean that this action is not determined by genes in the first instance. It is more significant that, except for a few rare exceptions mostly in the botanical realm, all self-perpetuating (i.e. hereditary) changes in cell constitution are genic in nature. Unless strong evidence to the contrary can be produced, it is safest to assume that all the properties of cells are ultimately determined by genes.

These considerations apply to the egg cell as much as to any other. The unfertilised egg is a product, and, in its finished form, a very late product, of the differentiation of its mother's body. Its properties must be mainly if not entirely determined by her genetic constitution. This is clearly seen in the few specific examples of egg differences which have been studied (e.g. left- and right-handedness in *Limnea*, certain characters of silkworm eggs, etc.).

In considering embryonic development, however, we are interested not so much in the ultimate determination of the characteristics of the developmental processes as with their immediate determination within the one life-history we are

¹ Fankhauser, 1929; Harvey, 1936.

studying. If we push our analysis no further back than the unfertilised egg, we halt at a stage at which the cytoplasm has already certain definite properties which are by no means without influence on the ontogenesis. We must therefore discuss the roles of the cytoplasm and nucleus of the fertilised egg; the discussion above should remove any suspicion that in so doing we are trenching on the question whether, in the final analysis, evolutionary importance can be attributed to cytoplasmic characters.

Within the limits of one life-cycle, then, we must first note that at least the earliest evocators seem to be cytoplasmic. An amphibian neural evocator can be extracted from the unfertilised egg by merely crushing and centrifuging, and there is no reason to doubt that this is the normal evocator. It is, perhaps, significant that the evocator is extremely unspecific in the sense that organisers can act on competent tissue belonging to a different order; chick organisers act in rabbits or newts, rabbit organisers in chicks, etc. However, certain substances which are produced under the action of genes during a single life-cycle are known to be quite widely distributed. For instance, the v^+ and cn^+ substances concerned in the formation of eye pigment in *Drosophila* (p. 78) occur in several orders of insects. Thus the wide distribution of the evocator is not in itself adequate grounds for supposing that it is cytoplasmic.

However, no example is known within the vertebrates of a difference in the neural evocator correlated with the specific nature of the animal from which it was obtained. In contrast to this, the competence of tissue is always strongly affected by its specific nature. It is well known that if an induction is made involving two species, the induced organ has the specific character of the competent tissue and not of the organiser;¹ at least this is true of all properties other than those which come under the heading of individuation, for which the situation may be more complicated. In the cases from which the experimental evidence of this dependence of competence on specific nature has been obtained, the specific differences have not yet been analysed in genetic terms. But there can be no doubt that the differences (or, if we wish to be over-cautious, the overwhelmingly greater part of them) are dependent on the kind and arrangement of the genes in the two species. Thus the available evidence, scanty

¹ Cf. Rotman, 1935, 1939.

though it is from the embryological side, shows that competence is very closely connected with processes directly controlled by the genes in that zygote.

Indeed, in general terms, such a conclusion seems inevitable on a *a priori* grounds alone. The evocator is merely a differential; it is the competence which is responsible for the details of the developmental process and thus of the kinds of tissue produced. Since it is the genes which control the characters of the animal and its tissues, it must in general be the genes which determine the properties of the competence.

Unfortunately, the animals which are most suitable for experimental embryological investigations are inconvenient for genetical analysis, at least of the developmental stages dealt with embryologically. There are therefore practically no data dealing directly with the effects of single genes on competence. Some gene-manifestations are undoubtedly suggestive in this connection. For instance, Whitlock¹ has described an eye defect in the guinea-pig, which looks as though it were due to a failure in the competence of the ectoderm to form a cornea, a process which is known to be due to an inductive stimulus, at least in the amphibia.² But in the absence of experiments, the interpretation remains in doubt: it is always possible that the fault lies with the inductive stimulus rather than with the competence. A very interesting case, in which the analysis can be pushed some distance, has been described by Bourne and Grüneberg.³ A recessive gene was found which caused a cataract of the lens, appearing in about the third month of life in the majority of homozygotes, although in a fair proportion of cases (40 per cent) it never appeared at all. Histological examination showed another effect, a degeneration of the retina, and this was found to be present in all homozygotes, both those which showed the cataract and those which did not; moreover it appears earlier than the cataract. Clearly the retinal degeneration is the more immediate effect, and is itself the cause of the cataract, presumably through some sort of continuous organising effect of the retina on the lens, which may remind one of the continuous effect of the retina on the growth of the lens in amphibia. The cataract effect, then, seems to be produced by altering, not the competence of the lens but the organising action

¹ Whitlock, 1935.

² See Mangold, 1931.

³ Bourne & Grüneberg, 1939.

of the retina. But one could go a stage further and say that the reduction in the organising action of the retina is a consequence of the retinal degeneration, which is itself to be regarded as an abnormality in the retina-forming competence.

Owing to our comparative ignorance of the effects of specific genes on competence, we must place more reliance on approaching the question from another angle, by investigating the effects of genes in general. We shall find that a discussion of gene action leads to the formulation of a scheme very similar to that which we have suggested from the embryological angle; that is, development will appear as a result of a complex of reactions between substances which form an unstable mixture, which may at certain times have two or more alternative modes of change open to it.

It is unfortunate that the paucity of our present data make it necessary to take many of the examples to be discussed from groups, such as insects, in which an evocator-competence mechanism has not been identified. But we have seen that there is no absolute difference between development depending on induction and so-called mosaic development. Both may be considered as manifestations of "developmental potencies" of the same fundamental kind, which are known as competences when they are dependent on an external stimulus. If we can obtain any insight into the way in which genes are concerned in determining the potencies of mosaic eggs, we probably shall not go far wrong in transferring these ideas to relations between genes and competences. At least, if we make any mistakes, they are inevitable, at the present time; the discussion in terms of mosaic potencies instead of competences is not a wilful confusion of the issues but is forced on us by the state of our ignorance.

CHAPTER VI

GENIC ACTION

THE mode of action of genes during development may be discussed from two quite different points of view. Firstly, we may attempt, from a consideration of gene action, to attain a deeper understanding of the mechanisms involved in differentiation. This is the issue with which we shall be mainly concerned in this book. But one may also investigate the developmental actions of genes in the hope of discovering, from the types of substances which genes produce, what the genes themselves consist of. So far it cannot be said that any very clear indications of the nature of the gene have been discovered in this way; probably the investigation of mutation and of the chemical nature of chromosomes is a more hopeful line of attack; but before proceeding to a discussion of the relevance of the genes to a general theory of development, it will be as well to consider briefly the considerations which have been advanced from this point of view.

The Nature of the Substances Produced by the Genes.

A survey of the literature will show that almost all known types of substance found in the animal kingdom may be influenced by genes. And if we believe, as most geneticists do, that every step of an animal's development is directly controlled by genes or was so controlled in the animal's ancestors, it is clear that there can be no limitation of the kinds of substances which as end-products can be affected by sequences of reactions set going under genetic control. The only question which it is interesting to ask in this connection is whether we can specify the nature of the substances which are immediately produced by the genes.

Goldschmidt¹ has drawn attention to the frequent production, under genetic control, of definite enzymes. Many more cases of this have recently been described; they are summarised by Haldane.² We may mention the various genetically controlled anomalies of metabolism, such as the lack of xanthophyll oxidase in yellow fatted rabbits, and the lack of a specific enzyme in alcaptonuria.

¹ Goldschmidt, 1927, 1938.

² Haldane, 1935.

But it seems an over-simplification to suppose, as Goldschmidt does, that all genes act by the production of enzymes. The intercalation of an enzyme between the gene and the antigen in the cases mentioned below merely adds to the story another element about whose nature we know not much more than we do about the other two. Similarly, knowing so little about the nature of the substances which interact with one another in the formation of a pattern, we are hardly justified in assuming that the genes can only control their formation through an enzymatic mechanism; indeed, if we persist in applying this hypothesis to all conceivable cases, the definition of an enzyme becomes so stretched that the hypothesis finishes by becoming completely meaningless.

Haldane¹ has drawn attention to the very direct relation which appears to exist between the genes for immunological characters and the actual substances by which these characters are manifested. For instance, if a man has the gene *A* his blood corpuscles will contain the agglutinin *A*, if he has the gene *B*, his corpuscles contain agglutinin *B*, if he has both genes, his corpuscles will contain both agglutinins. Similar relations hold for the immune bodies of other animals, such as fowls. It is tempting to suppose that this direct relation between gene and agglutinin indicates a real chemical similarity between them. But a direct relation of the same kind is also found between genes and certain other substances, which are not agglutinins. For instance, in the triploid endosperm of maize, the content of vitamin A is very nearly directly proportional to the number of *Y* (yellow endosperm) genes. The matter has recently been re-examined by Randolph and Hand,² who show that the proportionality, although very close, is not exact. Their figures are as follows:

	Diploid	Tetraploid
Cell volume	1	3.5
Carotenoids per unit of volume	1	1.45
Carotenoids per cell	1	5
Genes per cell	1	2
Genes per unit of volume	1.75	2.51
Carotenoids per gene	1	2.5

If evidence of this kind of a direct, or nearly direct, relation between quantity of gene and quantity of character is accepted as indicating the chemical nature of the gene, it will be necessary to

¹ Haldane, 1937.

² Randolph & Hand, 1938.

suppose that different genes are of quite different chemical constitutions.

Another method which may prove more valuable in the investigation of this question is the study of those cases in which the production of the gene-controlled substance follows very shortly after the segregation of the genes. For example, several factors are known which affect the chemical constitution of the pollen tubes in higher plants, and here the substances are produced in the short interval between meiosis and the growth of the pollen grain. In some of these cases also the substances may well be immune bodies; for instance, the numerous sterility genes determine an interaction between the pollen tube and the tissue of the style which might plausibly be envisaged in this way, although there is as yet no direct evidence on the point. But in other cases the substances would seem to be enzymes, as in the genetic control of the types of starch formed in the tubes.¹ Other examples in which there is only a short interval of development between gene and character can be found in some of the lower plants. Winge² has described the segregation in yeasts of genes which control the enzymes produced. Further work on such cases is much to be desired.

Finally, one must draw attention to the possibility that the direct production of substances by genes may be intimately related to the fundamental problem of gene reproduction. Koltzoff³ in particular has suggested that the molecule which a given gene produces may exist as such within the gene, may in fact constitute the whole of the gene except for a small protein part which enables the gene to attach itself to others and thus to make up a chromosome. This picture is probably unduly simplified. But it certainly seems the simplest hypothesis to suppose that the processes by which, between two cell divisions, a gene causes the appearance of a new similar gene, are related to the processes by which genes synthesise particular substances which affect development; and this again can be most easily imagined if one supposes that there is a considerable similarity in constitution between the gene and its primary product. The activity of a gene would then always be the same; it produces a certain substance, either free as an active substance, or bound as a gene in a chromosome.

¹ Cf. Brink, 1929; Darlington, 1937; Stern, 1938.

² Winge & Jørgensen, 1937, 1939. ³ Koltzoff, 1939.

Gene Effects.

In discussing the effects produced by genes during development we shall have to argue backwards from the manifest effects produced by genes in adult or late larval stages to the physiological processes which have occurred earlier. It is certain of many genes that they are concerned in several developmental processes. Many genes are known to have several directly visible effects, e.g. the white series of allelomorphs in *Drosophila melanogaster* which affect the testis colour as well as the eye colour. Often a slightly more detailed search reveals still other effects; the white series, for instance, also affects the viability and fertility. It is, further, often found that small patches of tissue from which a gene is completely absent (homozygous deficient patches) are inviable, even when the gene concerned cannot otherwise be shown to have any specific effect on that type of tissue.¹ From the point of view of a general theory of gene action, we should take it that all genes are concerned in all developmental processes; some perhaps with a zero efficiency in particular cases. When we argue, then, from the effect produced on a certain character by a given gene substitution, we shall be drawing conclusions only about one of the whole set of reactions with which those genes are concerned. We shall, however, speak of "the effect of the gene *A*" instead of, more correctly but more cumbrously, "that effect of the gene *A* which is relevant in the present context".

The reactions controlled by genes are reactions in a material system. The most typical reaction we can postulate is one which leads to the production of a specific quantity of a specific substance. It is possible, perhaps probable, that there are stages in the chains of reactions at which the effects produced by certain genes cannot be conveniently described in terms of the production of particular substances; a gene may, for instance, be effective at a certain stage by producing an alteration in pH , or in the permeability or polarisation of a membrane. For purposes of discussion, however, we shall in general neglect these possibilities and speak in terms of the production of substances; the other effects of genes are in so far similar that they are alterations in a material system, and an exhaustive discussion of the possibilities can be omitted here, where our purpose is to erect a general, not a

¹ Demerec, 1934.

particular, scheme of the relations between genes and the processes of development.

The gene effects which are most simply observable are the actual quantities and kinds of substance produced. A slightly deeper study may reveal the process of production of the substance. The term "gene effect" will in the following pages sometimes be used ambiguously, for both the substance itself and the process by which it is produced. In other contexts it is necessary to distinguish between these two concepts, e.g. in relation to the localisation of gene effects in space, since a substance may have a distribution different to that of its process of production.

It is convenient, in embryological discussion, to give the concept of substance a somewhat wider connotation than would be appropriate to chemical theory, and this is also frequently done in genetics. Thus we find discussion of an ommatidia-producing substance which is controlled by the Bar gene in *Drosophila*. Clearly the production of ommatidia is not the function of a single substance; but on the other hand the number of ommatidia in an eye is a measure of the quantity of a single sort of stuff, namely ommatidia. We can always postulate an underlying constellation of substances, which produce ommatidia when, and in proportion as, they are acted on by a hypothetical ommatidia-stimulating substance. In some cases, where organiser phenomena are known to be concerned, there is good reason to argue in this way, but in general it is better not to make specific hypotheses as to the mechanisms at work in the production of a certain quantity of tissue; any such hypothesis will at best refer only to the last two or three stages in a long chain of reactions. We can instead expand the idea of substance to cover any entity which is divisible into similar units whose number we can estimate quantitatively. Thus we shall, on occasion, speak of a tissue as a single substance, whose quantity can be measured by its mass; similarly, in connection, say, with Stern's¹ work on bobbed, there is no danger in speaking of the length of the hairs of *Drosophila* as the measure of a substance. But it must be remembered that by this usage we postulate neither a single hair-precursor substance, quantitatively proportional to the amount of hair produced, nor, of course, that the hair is a simple chemical substance.

¹ Stern, 1929.

Substance and Pattern.

We have said above that the most easily observable type of gene effect is an alteration in the quantity or kind of substance produced. There are, of course, many genes whose action cannot as yet be described in such terms. There are gene effects, for instance, which are psychological in nature, others which are expressed as susceptibilities to disease, etc. In such cases we know so little even about the material nature of the end-effect of the reaction sequence controlled by the genes that it is hopeless to attempt a discussion of the nature of the reactions themselves. Such difficult cases must be left on one side for future investigation.

There is one class of gene effects, not easily conceived of in terms of kinds and quantities of substances, which is, however, of quite crucial importance for the study of gene reactions in development. These are the pattern effects of genes. The effect of the polydactyly gene, for instance, cannot be described in terms of the quantity or kind of substance which it produces, but only in terms of the pattern in which the digits are arranged; the same is true of the numerous colour-pattern genes which are known. The importance of these genes lies in the fact that pattern formation is perhaps the most fundamental achievement of a developing embryo; and it is certainly the most mysterious. If we wish to be able to show how genetic factors control development, even if we only aim at giving an abstract theoretical scheme which shows the kinds of processes which will have to be considered, pattern formation is an essential factor which cannot possibly be omitted. The only clue to the understanding of the genetic control of the major morphological patterns of the animal body must be provided by the study of the pattern-producing genes we know, which so often affect only trivialities such as colour patterns.

The discussion of the patterns produced by genes will be taken after the discussion of the production of substances. In the first place, it is perhaps more logical to discuss the production of substances before we discuss how the substances are arranged; secondly, we know much more about the production of substances; and finally, it is necessary to make an attempt to exhibit the control exerted by genes over patterns as a particular result of the production of substances. A pattern, after all, is an abstract concept; and a gene must produce some material thing. In the

days when the gene was itself an entity defined purely in biological terms, by reference to numerical ratios in crosses, we might be content to correlate it with an effect described also in biological terms as a pattern. But with the much deeper knowledge which we now possess of the material nature of the gene, there should go a more material description of the effects. We may defer the attempt when confronted with genes with psychological effects, but we cannot refuse the challenge in respect to the major half of the whole ontogenetic process; but we must be ready to find ourselves confronted with the age-old problem of translating morphological descriptions out of geometrical terms into the language of the physical and chemical interaction between material bodies.

The Localisation of Gene Effects.

Embryonic development cannot be discussed solely in terms of temporal process; spatial considerations are equally, perhaps more, important. A preliminary classification of the kinds of localisation of gene effects can be given as follows:

(a) Immanent.

By an immanent gene effect is meant one which is part of the general constitution of the unfertilised egg. Such an effect is one of the fundamental elements in the whole subsequent development of the zygote, and must be supposed to have some relevance to every reaction which occurs in it. The most famous gene substitution with an immanent effect which has been studied is that controlling right- and left-handedness in *Limnea*. The type of symmetry of the embryonic cleavages and of the adult spiral is in this case determined by the genetic constitution of the mother. The effect of the gene skips a generation and is seen in the offspring, which may or may not possess the dominant which has come to expression. For a given individual, its mother's genes, which are effective in it, are immanent in the sense that they pre-condition its whole geometrical system of development. Most cases of maternal inheritance, such as the handing on through the egg of a supply of pigment, or the determination by the character of the egg of the rate of cleavage, are to be regarded as immanent effects. They must be taken to fall into this category, as regards their localisation, quite regardless of the time in the maternal life-cycle when the gene-effect was actually produced. For instance,

Caspari¹ has shown that the substance set free in the blood by the gene *A* in *Ephestia kuhniella*, which causes the darkening of the eye pigment (p. 76), is handed on by a mother which possesses it to her offspring, and that the mode of transmission is through the cytoplasm, not by an action of the oocyte nucleus. Thus the substance was transmitted to the offspring of homozygous recessive *aa* mothers in which the substance had been produced by transplanted *AA* tissue. The localisation of the *A* effect in these offspring must be classified as immanent. It should be noted that the supply of *A* substance transmitted in this way is inadequate to produce dark eyes during the whole life of the offspring, but only affects the larval stages.

(b) Pervasive.

A primarily pervasive gene-effect is one which is produced in every cell of the organism. The emphasis here is on "produced" we shall mention in a separate category effects which are produced in a localised area and spread from there over the whole animal. It is difficult to find examples of primarily pervasive effects in the animal kingdom, but probably the genes which determine the serological specificity of animal species belong to this category. In plants the genes controlling the production of chlorophyll are almost completely pervasive, although even they may have no expression in certain organs such as the flower and root.

(c) Localised.

Gene effects which are not pervasive must be localised, and this is much the most important category of effects. We shall have to consider subdivisions:

(1) Strictly localised.

These are the substances which are produced in a single place, or specified places, and do not diffuse out into the surroundings. Most of the end-products of gene reactions, which are the products we actually see, are probably strictly localised. Thus the eye pigments of *Drosophila* are produced in the eye and stay there. Even the pigments of skin in a piebald animal probably do not diffuse as pigments. The question of the strictness of localisation becomes much more interesting however, and much more difficult to determine, when we consider the intermediate products of gene

action, the pigment precursors, for instance. The presence or absence of these can usually only be proved by experiment. Thus it was only with the development of the transplantation technique by Ephrussi and Beadle that we learned of the diffusible pigment precursors concerned in *Drosophila* eye pigmentation (p. 76). Indeed, if we follow back the chain of reactions which leads to the localised production of a gene effect, it will always be difficult to prove that the primary effect of the gene in this series has been a localised one, since there is always the possibility that an apparently local effect is due to a local reaction to an alteration in general conditions. At the same time, the local reactivity must itself be under the control of other genes, so that there can be no doubt that there must be genes whose effects on a certain series of reactions are strictly localised; the difficulty is to prove which these genes are. This uncertainty greatly increases the difficulty of discussing the localisation of gene action, since we have no quite secure examples from which to start.

(2) Diffusely localised.

These are the substances which are produced locally, but spread from their point of origin for a short distance, through the tissues. The category does not include substances which escape from their place of origin into the blood stream or body fluid and permeate the whole system (see next category). A good example of a diffusely localised gene-effect has been described by Whiting;¹ in mosaic-eyed individuals of *Habrobracon*, substances are produced by the darker regions, containing the dominant allelomorphs of the orange series, which diffuse into the adjacent light areas containing the recessive allelomorphs and bring about a darkening in colour. The sex substances discovered by Witschi² in the Amphibia (corticin and medullarin) are also diffusely localised substances in most groups, though in the frogs they may be permeating substances, since they seem to spread through the whole body. The distinction between diffusely localised substances and permeating substances is, as this example shows, probably not a very fundamental one. The reason for introducing a separate category for the diffusely localised substances is their great theoretical importance. Embryonic evocators are diffusely localised substances.

¹ Whiting, 1934.

² Witschi, 1934.

(3) Permeating.

These are substances which are produced locally but spread from their place of origin until they permeate the whole body. Examples are the sex hormones, or the substance responsible for the dwarfism of mice with heredity defects of the pituitary.¹ Both these are gene-produced substances which become permeating and which themselves probably produce some effect in every part of the body. There are probably many permeating substances whose effects are localised because they only react in certain sensitive areas. Thus the vermilion and cinnabar substances in *Drosophila* are produced in only a few organs; the vermilion substance in the fat bodies, Malpighian tubules and eyes, the cinnabar substance in Malpighians and eyes.² From these sources they reach the blood stream, and become permeating, but their effects are again localised, in the eyes and the ocelli.

The Interaction of Gene Effects.

If a gene-produced substance appears in a localised area, whether by a local reaction to a permeating stimulus or in any other way, it is still not quite easy to discover whether it is strictly or diffusely localised. This can only be determined if there is in the neighbourhood other tissue which does not itself produce the substance, but which could react to the presence of the gene substance in question, if it should diffuse into the area. This condition must often occur in normal development, but usually the gene substance will diffuse throughout the whole mass of reacting tissue, so that the whole of this tissue shows a uniform character which obscures the fact that diffusion has been at work. The necessary information can be obtained in certain cases of abnormal development, both spontaneous and experimental. We may get areas of similar tissue in the same animal which differ in their genetic constitutions, and in one of these areas a gene substance may be produced which can diffuse out and influence the character of the other area where the genetic basis for the production of the substance is absent.

This condition occurs spontaneously in mosaic individuals, formed either by somatic mutation, somatic elimination of a chromosome, or double fertilisation. Sturtevant³ showed that in

¹ Smith & MacDowell, 1930.

² Beadle, 1937.

³ Sturtevant, 1929, 1932.

Drosophila melanogaster the presence of eye tissue containing v^+ prevented the expression of vermilion in areas homozygous for the mutant gene; that areas of B^+ eye tissue exert a facet-forming influence on nearby B eye tissue; and that y^+ surroundings darken patches of yellow tissue. For many other genes, on the other hand, tissue containing the normal allelomorphs exerts no effect on neighbouring mutant tissue. We clearly have here to deal with two variables, the diffusibility of the gene substances and their possibilities of interaction. A scheme of classification of diffusibility has already been provided. For the possibilities of interaction between the products of any pair of genes, we have the three possibilities to consider, that the presence of one gene affects tissue containing the other, or that it does not affect the other, or that both areas with the different genes affect one another mutually. In the first case, where there is an effect, it is analogous, among gene substances, to the phenomena of dominance or epistasis between genes. The gene substance which produces the effect will be said to be *eparchic*, that on which the effect is produced *hyparchic*. These terms apply strictly to the gene substances which actually interact, but where the context is sufficiently explicit it is probably more convenient and permissible to speak of one gene as eparchic to the other. Where there is no effect of interaction, the genes (strictly the gene substances) have been said to be "self-differentiating" (e.g. Sturtevant). This term, however, is quite sufficiently ambiguous even when used in reference to experimental embryological facts where it was first invented, and it is probably a mistake to extend its use to the rather different meaning given to it here. Less objection can be taken to the term "autonomous", which is also used in this connection, but it does not lend itself very readily to the formation of the other words required for the other possibilities; in this book the word "autarchic" will be used of a gene (or gene substance) where expression is not affected by substances reaching it from its surroundings. Finally, for the case of mutual interaction, we may coin the term "synarchic".

Some interesting mosaics have been described¹ in the parasitic wasp, *Habrobracon*. The mosaic individuals are haploid males, which have arisen by double fertilisation of the second polar body as well as the egg nucleus. Two eye-colour genes, cantaloup and white, have as yet proved autarchic both as regards their normal

¹ Whiting, 1934; Whiting & Whiting, 1934.

allelomorphs and all other genes. The allelomorphs of the orange series, however, produce diffusely localised gene substances which show clear effects of interaction. Thus in eyes mosaic for ivory (o^i) and either of the darker allelomorphs black (O) or orange (o) there is no sharp boundary between the colours; one could assume

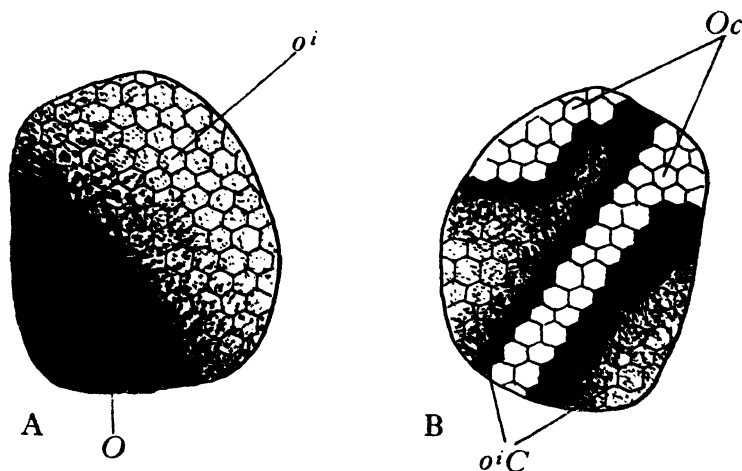


Fig. 5. Mosaics in *Habrobracon*. A is a right eye, the bottom left part of which contains the dominant factor for black (O), while the upper right has the recessive allelomorph ivory (o^i); note how the dark colour spreads from the black area through an intermediate zone, which is really reddish orange, into the pale ivory region. B is a left eye which contains two areas which are black cantaloup (Oc); since the latter is epistatic, these are pale in colour. The remainder of the eye is ivory normal-cantaloup (o^iC). These areas should also be pale in colour, but a substance spreading from the Oc area causes them to be black at the edges, grading through orange to ivory. (After Whiting.)

either that the dark colours are eparchic to ivory, or that the two are synarchic. The gradation between black and ivory gives an intermediate orange eye colour which is apparently the same as that produced by the intermediate allelomorph orange; the simplest explanation is clearly that black, orange and ivory differ only quantitatively from one another in their effect on the pigment-producing system. Some other mosaics make it very probable that actually black is eparchic to ivory and orange rather than that they are synarchic. Black O is not shown in haploid tissue

which also contains the recessive cantaloup c , which is epistatic. Thus in mosaics for Oc against o^iC , the Oc facets are pale cantaloup in colour. They are found to form an area with a perfectly sharp border, and immediately against them occurs not ivory (which can be shown in the presence of C) but black, which is clearly produced by the diffusion from the Oc area of an eparchic substance produced under the influence of O ; this substance cannot be the pigment itself, but must be some precursor whose utilisation in the Oc area is prevented by c .

The production of the O substance in *Habrobracon* is as a matter of fact not confined to eye tissue, but also occurs in the testes; and in mosaic individuals with O testes and o^i eyes, the eye colour is darkened. Thus the substance is localised in at least two places and is, at least potentially, permeating. Other similar phenomena have been studied in *Drosophila* and *Ephesia* and are discussed later (p. 76).

CHAPTER VII

THE TEMPORAL COURSE OF GENE REACTIONS

Time-effect Curves.

GOLDSCHMIDT¹ was the first to lay emphasis on the importance of studying the temporal course of the reactions set going by genes. He described the gradual production of pigment in the caterpillars of *Lymantria*, and he also used a hypothesis of this type in his well-known theory of the physiology of intersexuality, which was founded on his studies of the same moth. He coupled the observation or induction of temporal processes of production of substances with a theory that the genes controlled these processes by virtue of being enzymatic in nature, and was thus led to suppose that the reactions which are immediately controlled by the genes must have a linear course when plotted against time. This part of his theory is an addition to the basic insistence on the importance of temporal aspect of gene reactions, which remains unaltered whether the further hypothesis is true or not. Actually, it is difficult to maintain consistently that all gene reactions follow a linear course; even Goldschmidt, in explaining dominance for example, is forced to consider the interaction between the products of a linear reaction and some other reaction which is not linear, but which must presumably be also under genetic control. We shall here make no assumption as to the linear or non-linear character of the gene reactions, but shall usually represent them as non-linear, for the sake of generality. It is certain that the reactions whose results we can directly observe, which are probably always the last link in a sequence of reactions, can often be shown by direct observation to be non-linear; a fact which Goldschmidt would not dispute.

The curve obtained by plotting the amount of end-product produced against time, or time-effect curve, has been exactly determined in only a few cases. One of the best is the development of eye pigment in the amphipod *Gammarus chevreuxi*.² In this case we obtain a family of curves which reach different

¹ Cf. Goldschmidt, 1927.

² Ford & Huxley, 1929.

asymptotic values, or approach the same asymptote at different rates. In many animals, such as *Drosophila*, most of the processes of development cannot be observed at all easily, and our knowledge is confined to the final state, which may be an asymptotic value, or may be some intermediate point on the curves, determined by the cessation of development on emergence from the pupa, for instance. We can in such cases still derive some information about the family of curves, though not about each individual curve, by considering the relation of the different asymptotic or limiting values and the dosage of the genes. Stern¹ was

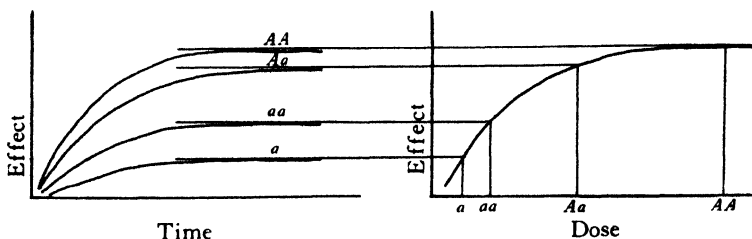


Fig. 6. The relation between time-effect curves (on the left) and dose-effect curves (on the right). (From Waddington.)

able to study the length of bristles in *Drosophilae* containing various numbers of bobbed genes, adding the bobbed genes in fragments of the *Y* chromosome, which are known to be otherwise more or less "empty", so that they do not upset the general genotypic balance. From his data he plotted a dose-effect curve, showing length of bristle against the quantity of gene. From such a dose-effect curve we can construct a set of hypothetical time-effect curves, where the actual course of each curve is uncertain, but the asymptotes (or limiting values when development ceases) are fixed.

If a dose-effect curve is set up for various doses of one allelomorph, the data relative to another allelomorph can be fitted in, and this makes it possible to fix the relative dosage strength of the two allelomorphs. In general it is found that the normal allelomorph has a strength considerably greater than that of the mutants. Muller² has proposed that genes which differ from their normal allelomorphs only in their dosage strength should be called

¹ Stern, 1929.

² Muller, 1932.

hypomorphs if they are weaker, hypermorphs if they are stronger, than the normals. These terms, like dominant and recessive, or epistatic and hypostatic, are of course relative and can strictly only be applied to one of a pair of genes. In *Drosophila*, where there is a well-defined wild type, it is natural to take the normal allelomorph as the standard, but in other organisms where the wild type is less obvious it is meaningless to say that a certain allelomorph is hypomorphic unless one states which other allelomorph it is hypomorphic to. It is convenient to have a word for a set of genes which are hypomorphic or hypermorphic to one another, and they will here be referred to as a set of quantity genes.

The course of a time-effect curve is not determined solely by the gene whose name is attached to it, but is the result of the combined action of that gene and the whole of the rest of the genotype. The effect of some modifying factors on the time-effect curves of *Gammarus* eye-pigment genes has been described, but we have rather little exact information on other cases. The prevalence and importance of such genotypic modification of the time-effect curves can, however, be deduced from the effects which the genotypic milieu is observed to produce on the dose-effect curves, since, as we have seen, a modification of this curve must be a result of the shifting of the asymptotes of the time-effect curves. Thus it has been proved for several genes that in setting up a dose-effect curve it is necessary to consider not the absolute, but rather the effective dose, measured by the relation between the absolute dose and the quantity of the rest of the genotype. Thus Schultz¹ showed that the effect of a given dosage of shaven (a IVth chromosome bristle gene in *Drosophila melanogaster*) is less in the triploid than in the diploid. The same thing is, as is well known, true of the sex genes. In both cases the effect is controlled by the relation or balance between the particular genes and the rest of the genotype, and this is of course evidence that the dose curves, and hence the time curves, are properties of the whole genotype.

The main evidence of the influence of the genotypic milieu on the dose-effect curves comes from the related phenomena of the evolution of dominance and dosage compensation. It is well-known that the normal allelomorphs, in animals which have a well-defined wild type, are usually dominant over the mutants. Very

¹ Schultz, 1934.

many of the genes concerned are quantity genes, and the dominance is to be explained in terms of hypermorphism. If the normal allelomorph A is dominant over the mutant a , the effect of the heterozygous combination Aa is the same as that of the stronger combination AA and must therefore lie on the horizontal part of the dose-effect curve. Now Fisher¹ has suggested that this is brought about during evolution by the gradual alteration in the relative effective strengths of A and a , caused by the modification, under natural selection, of the genotypic milieu. He supposes that when the mutation a first occurs, the effect of the compound Aa is likely to be intermediate between those of AA and aa ; in such a case AA must be somewhere near the top of the bend of the dose-effect curve. Owing to the selective advantage of those genotypes containing Aa which phenotypically approach nearest to AA , Fisher suggests that the general genotypic milieu of the species is altered in such a way that Aa comes always to have the same effect as AA . This involves the raising of the dose-effect curve between aa and AA until the stretch between Aa and AA is horizontal. Fisher postulates then a genotypic control of the dose-effect curve, and this necessarily involves the genotypic control of the time-effect curves. Muller² has drawn attention to the other possible way of producing dominance of A over a ; we can arrange that Aa and AA shall both lie on the horizontal part of the dose-effect curve, not by raising the early part of the curve, but by lowering the later part, and Muller suggests that in the evolution of dominance the prevention of AA from having too great an effect may be just as important as ensuring that Aa has enough. This mechanism, like that described by Fisher, also involves the genotypic control of the curves, and is thus indirect evidence, for the point which we are discussing, namely the collaboration of the whole genotype in the control of the time-effect curves. Haldane³, however, has suggested a method of dominance evolution which is independent of genotypic control of the dose-effect curves. Taking this curve for granted, i.e. not discussing the factors which determine it, he points out that it will be advantageous to a species to adopt as the wild type a gene whose homozygote lies some way along the horizontal part, which will automatically ensure that the heterozygote with slightly hypomorphic mutations will lie on the horizontal part also. This

¹ Fisher, 1928, 1931.

² Muller, 1932.

³ Haldane, 1930.

mechanism, while not directly postulating anything about the genotypic control of the curve, is of course not incompatible with such a hypothesis.

Other evidence for control of the dose-effect curve comes from the phenomenon of dosage compensation. Stern¹ and Muller² drew attention to the fact that a female homozygous for a recessive sex-linked gene usually shows much the same phenotype as a male containing only one dose of the recessive. This can only be understood if we suppose that the relative or effective dosage of one gene in the male is the same as that of two genes in the female. This can only be so if the other part of the genotype against which a sex-linked factor is balanced is the rest of the *X* chromosome, which, like the factor itself, is present in double dose in a female and single dose in a male. The important point for the present context is that dosage compensation of sex-linked factors is very easily observed and a very common phenomenon, and thus shows that the genotypic control of the dose curve is not peculiar to a few genes but is characteristic of the great majority, if not all, of them. The evolutionary reasons for the production of a dosage compensation mechanism do not concern us here; they have been discussed by Muller, who suggests that the fundamental point must have been the evolution of dominance of the wild allelomorph in females.

Developmental Stages.

We have now arrived at the concept of the time-effect curve as something which is implicit in the genotype as a whole; we can picture the curve as modelled by the actions of the whole complex of factors, some of which tend to push it upwards at a certain time, while others are holding it down. The net result is a process following a definite course in time. This concept now requires widening in two respects.

Firstly, the actual time-effect and dose-effect curves on which the theoretical discussion has so far been based relate only to the final visible end-products of the sequences of gene reactions. Clearly, however, there will usually be preparatory earlier reactions which lead up to the inception of the reaction whose visible products we see. These preliminary reactions must also follow defined courses, since the constancy of the end-reaction presupposes a constancy

¹ Stern, 1929.

² Muller, 1932.

of the earlier stages in the sequence. On theoretical grounds, then, we are justified in thinking of a time-effect track, extending the concept of the curve to cover the earlier stages in the sequence, whose products will not be of the same nature, and cannot strictly be plotted on the same co-ordinates as those of the end-reaction.

Secondly, this concept of a time-effect track must be widened to cover the production by genes of qualitative as well as quantitative variations in the substances formed. Scott-Moncrieff¹ and others have, for example, investigated the different types of anthocyanin pigment formed in flowers, and shown that genes control the synthesis of several different molecular patterns. In this case it is conceivable that some of these differences are directly produced by the genes, if we can suppose that the genes themselves react with the anthocyanin precursors. But in general it is very improbable that the substances in which the differences are finally manifested are immediate products of genes. Genetic control of the kinds of substances produced usually involves, as a first step, an effect on some intermediate product which forms part of a sequence of developmental reactions. By an alteration at an early stage in the sequence, the whole course of the later reactions is changed. We may say that the time-effect track branches at this intermediate stage, and the gene controls the choice of which branch shall actually be followed.

It is not easy to discover the nature of the intermediate stages in the gene reaction sequences. By analysing a very large number of compounds, Wright² and his students have been able to formulate a hypothesis of the actions and interactions of several colour factors in the guinea-pig, which is not only qualitatively satisfactory, but can also be made a basis for quantitative prediction. It is rarely, however, that the genetic material available is rich enough for this method of attack to be successfully pursued; another example, though less complete, is provided by the investigations of Mainx³ on combinations of eye-colour genes in *Drosophila* (p. 78).

One might expect that genes whose expression is known to be affected by environmental conditions would be more favourable material for a study of the reaction sequence. The important investigations on the formation of pigment in the Himalayan

¹ Scott-Moncrieff, 1936, 1937.

² Wright, 1940; E. S. Russell, 1939; W. L. Russell, 1939.

³ Mainx, 1937.

rabbit,¹ which is white except for black patches on the extremities, started from the observation of Schultz that black hairs can be formed in the usually white areas if these are kept at a low enough temperature. The critical temperature, above which pigment is not formed, varies somewhat in different races, and is also correlated with the heat-regulative capacity of the animal. It is about 33° C. for homozygous Himalayans (c^nc^n) and slightly lower, about 31° C. for the heterozygote with albino (c^nc). These temperatures apply to the skin itself, not to the underlying tissues. It next appeared, however, that these temperatures are not critical for the actual production of pigment, which can be formed in skin at a considerably higher temperature (up to 45° C.), provided this skin has been previously cooled below the critical temperature for a sufficient length of time. The "undercooling" phase is therefore concerned with some preparatory process which must precede the actual pigment formation. Further, a still earlier phase can be distinguished by a study of the effects of X-rays, which seem to prevent the production of some substance which is necessary in the undercooling phase: a piece of skin which is undercooled shortly after X-raying can always form some pigment, presumably from a store of "undercooling substrate" which is already contained in it, but no more pigment is formed if this same piece of skin is undercooled a second time after an interval of some days. The point at which the albino alleles enter into this sequence is indicated by the fact that the minimum length of time of undercooling necessary for pigment formation is less for c^nc^n than for c^nc , whereas of course Cc^n forms pigment even at higher temperatures; the genes therefore effect the undercooling phase.

The analysis of this sequence of events has even progressed to the stage of being able to identify chemically some of the processes concerned. The final pigment formation is the production of a melanin by the action of an oxidating enzyme on dioxyphenylalanine and is inhibited by cyanide and anaerobic conditions. The undercooling phase, on the other hand, is not dependent on oxidations, and consists in the formation of the oxidase. The nature of the X-ray sensitive phase is unknown.

Some information as to the chemical systems involved in pig-

Daneel, 1934, 1937, 1938; Engelsmeier, 1935, 1937; Schultz, 1916, 1935; Daneel & Labnow, 1937; Daneel & Schaumann, 1938.

ment formation has also been derived from direct extraction experiments. Thus Onslow¹ showed that extracts from the skin of agouti and recessive black rabbits contain a tyrosinase which can form melanin when mixed with Dopa (dioxypyphenylalanine), whereas recessive white skin does not. Dominant white skin, again, contains an inhibitor which prevents the reaction between Dopa and the tyrosinase from recessive coloured skin. Finally Koller² showed that dominant black skin contains an inhibitor which inhibits the inhibitor from dominant white skin, and therefore allows pigment to be formed.

Another good example of the analysis of the sequence of reactions leading from the gene to the adult character is provided by the investigations of Beadle and Ephrussi³ and others on the eye-colour system in insects, where advantage has been taken of the lack of strict localisation of gene-effects, and lack of autarchy, to discover the course of the intermediate reactions.

Caspari⁴ introduced the method of transplantation of imaginal discs between larvae of different genetic constitutions. He worked in *Ephestia*, and was able to show that the production of the normal pigmentation of the eye requires a substance manufactured by the dominant gene *A*, which is lacking in *aa* genotypes unless supplied to them by transplanted *A* tissue; this tissue might be either eye or gonad, so that the production of the permeating *A* substance occurs in at least two localities. Beadle and Ephrussi adapted this technique to *Drosophila*, and, with the much greater range of different genes available in that species, obtained much fuller results. They could demonstrate three successive stages in the sequence of reactions leading to normal pigment formation. The first stage which was revealed in their experiments required the presence of some substance dependent on the presence of the normal allelomorph of claret; in the absence of this the reactions proceed along a course which leads to the formation of a claret-coloured eye. The substance is not manufactured in the eye itself but in some other part of the body, from which it passes into the eye during the period between 80 and 106 hours after egg laying; if a wild-type eye disc is implanted into a claret host before this period, it receives no *ca*⁺ substance and develops a claret colour,

¹ Onslow, 1915.

² Koller, 1930.

³ Beadle, 1939; Becher, 1938; Ephrussi, 1938, 1939.

⁴ Caspari, 1933.

while if it is implanted from an older donor it already has its supply of the substance and becomes wild type in colour. A claret eye disc transplanted to a wild host does not become wild in colour, so that the claret gene seems not only to prevent the formation of the ca^+ substance but also to prevent its use if it is supplied.

As well as the ca^+ substance, two other substances have been found which are involved in the wild-type pigment sequence. These are the substances formed by the normal allelomorphs of

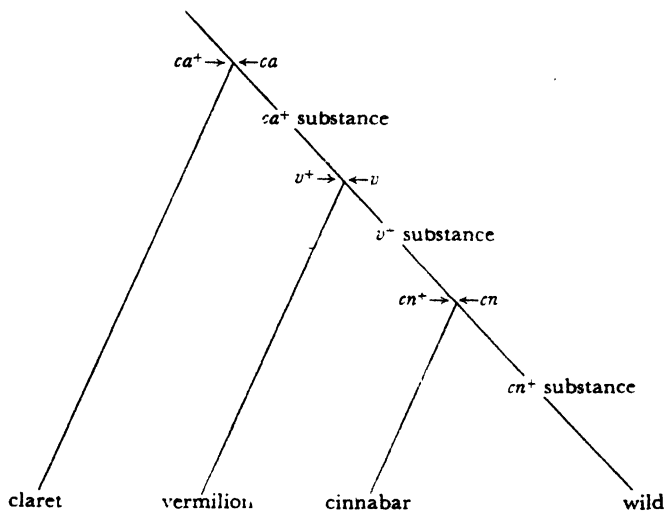


Fig. 7. The formation of eye colours in *Drosophila*. The pigment-forming process normally runs down the line through the ca^+ substance, the v^+ substance, and the cn^+ substance, to give wild type pigment. The genes, ca , v and cn interrupt this sequence, so that the process takes an altered course, to give claret, vermillion or cinnabar pigmentation.

vermillion and cinnabar. If eye discs of either of these types are implanted into normal hosts, they develop wild-type pigment; thus both the substances are permeating and can diffuse to the implanted eyes. By injection of wild-type lymph containing v^+ substance at different ages it was shown that this substance must be absorbed before the 70th hour after pupation. The v^+ substance seems to be a precursor of the cn^+ substance, since a vermillion disc implanted into a cinnabar host becomes wild in pigmentation; it presumably receives from the host the v^+ substance which it cannot make itself and is then able to manufacture its own cn^+

substance. A cinnabar disc implanted into a vermilion host already makes its own v^+ substance but receives from the host no help in making the cn^+ substance and therefore remains cinnabar in colour. The relation between the ca^+ substance and the v^+ and cn^+ substances is obscure; Beadle and Ephrussi originally suggested that it was a precursor of v^+ , since the evidence then indicated that a claret fly contained neither v^+ substance nor cn^+ , but this result is now regarded with some doubt, and the question cannot as yet be decided. Accepting provisionally their earlier hypothesis, we can postulate a series of changes by which ca^+ substance is converted into v^+ , and that into cn^+ . A purely diagrammatic representation of the track of the reaction sequence in which these substances are concerned is shown in Fig. 7. The track is of course a branching one; if v^+ substance is formed, the pigment system can develop a cinnabar pigment, if not, then it must develop along the track to vermilion pigment, and so on.

We do not yet have much information as to the actual chemical nature of the substances concerned in this chain of reactions or of the pigments finally produced. Mainx¹ has shown that there are at least two pigments in the *Drosophila* eye, a red water-soluble one, and a yellow or brownish one which is insoluble in all usual solvents. The yellow pigment is the first to be laid down, and it is probably mainly with this component that the vermilion and cinnabar substances are concerned. The direct investigation of the chemical nature of these two substances has already proceeded some distance.² Both are soluble in water and alcohol, not in ether or chloroform. They are stable at 100° C. for an hour, but are destroyed rapidly at 160° C. Their solubilities and precipitation reactions suggest that they are amino acids, but the comparative lack of stability to heat, and the instability in the presence of strong acid or alkali, makes it likely that they are complex rather than simple amino acids. A preliminary estimate of the molecular weight of the vermilion substance, by the diffusion method, gives a value of between 400 and 600.

From an evolutionary point of view, it is very interesting to find that these substances are not by any means confined to *Drosophila*.³ They can be extracted from other Diptera, such as *Calliphora*, and from Lepidoptera such as *Ephestia*. Indeed, in the latter animal,

¹ Mainx, 1937.

² Tatum & Beadle, 1938.

³ Beadle, Anderson & Maxwell, 1938; Becher & Plagge, 1937.

it has been shown that the *a* gene is homologous with the *v* of *Drosophila*, and leads to the absence of a substance identical in its developmental properties with the vermilion substance. A similar homology can be traced even in a group as far removed as the Hymenoptera; the *o* series of allelomorphs mentioned above (p. 67) seems to be homologous with the *Drosophila cn* gene.

This work is a very promising beginning in the identification of the substances intermediate between the gene and the final pigment. Much further work will be necessary before this gap can be fully filled in.

The Branching-Track System.

In the examples we have just considered, the formulation of the developmental reactions as a set of branching tracks, suggested above (pp. 74, 77), would be rather a clumsy method of expression. Although we might legitimately say that in the absence of the *v*⁺ substance the pigment precursors continue their development along an abnormal path, it will probably appear to the reader that we should not gain any further insight by such a statement; probably all that happens in fact is that in the absence of the *v*⁺ substance, the synthesis of the brown pigment fails to occur. The formulation as a branching track system only becomes necessary when a considerable amount of differentiation occurs subsequent to the point of branching. We have, in fact, been discussing the developmental processes which make up particular competences, but we have still to turn our attention to the genetic data which indicate the existence of alternative modes of development.

As an example in which a formulation as a branching-track system is more appropriate, we may consider the genetic control of the development of the antenna in *Drosophila melanogaster*.¹ In the wild-type fly, this organ consists of a small basal joint, a somewhat swollen second joint, and a third joint which bears the arista, a tapering chitinous spike having numerous branches. The recessive mutation *aristopedia* *ss*^a causes a profound modification.² The arista is transformed into a more or less leg-like organ, consisting of several small joints resembling the tarsal joints, the terminal one of which usually bears two typical tarsal claws; at the same time the third antennal joint is usually less swollen and more elongated than normal. There is then a branching in the

¹ Waddington, 1940 *a, b*.

² Balkaschina, 1929.

developmental possibilities open to the antennal imaginal bud; it may follow one branch and proceed to develop into an antenna and arista, or the other and develop into a leg-like organ.

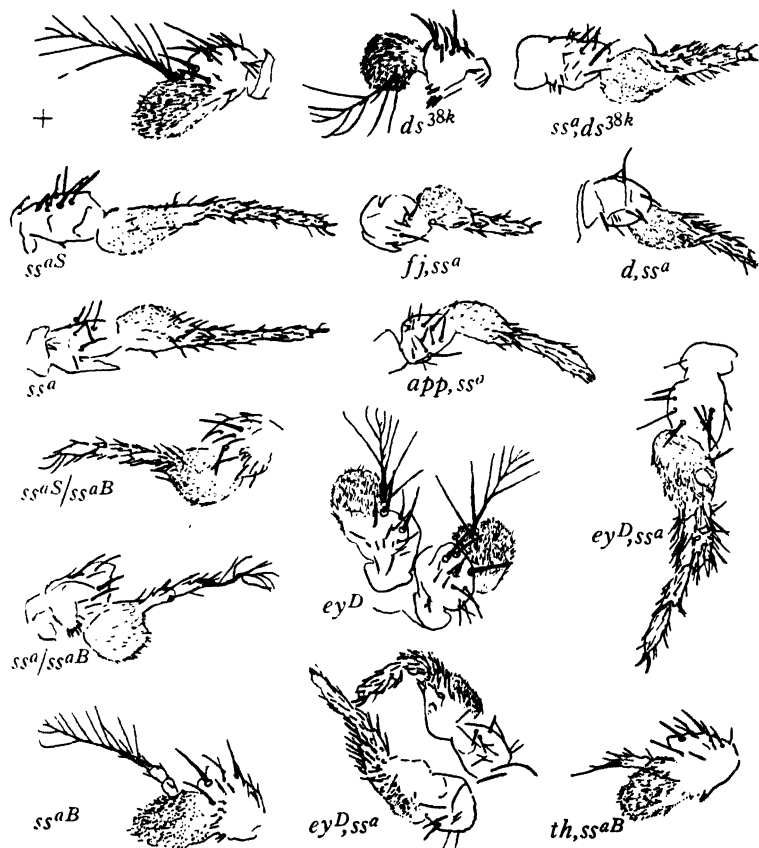


Fig. 8. Antennae of the different allelomorphs of *aristopedia* and of some compounds. The ey^D and ey^D, ss^a at the lower centre are duplicated antennae from one side of the head.

The mechanism of this branching, and the time in development when it occurs, are unknown. It certainly happens in an early larval stage, and the ss^a antennal buds cannot be affected by diffusible substances in the body fluids of normal host larvae, since they

develop as tarsal organs after transplantation into wild-type larvae¹. Some information about the branching may be obtained from studying the different allelomorphs of aristopedia. The whole group of alleles at the locus has three main effects: (1) on the length of the bristles, most marked in *ss*, which has no apparent effect on the antenna, (2) on the development of the antenna, (3) on the development of the legs. Here we are only concerned with the second effect. There are three alleles which produce the effect in fairly marked degree, *ss^a* aristopedia, *ss^{a-Sp}* aristopedia-Spencer and *ss^{a-B}* aristopedia-Bridges. Of these, *ss^{a-Sp}* is the "strongest"; the arista are completely tarsus-like and the third antennal joints considerably elongated. Aristopedia-Bridges, *ss^{a-B}*, on the other hand, has a comparatively weak effect; often it produces only a slight thickening of the base of the arista. In compounds of the alleles, e.g. *ss^{a-Sp}/ss^{a-B}*, the genes behave as though they differed quantitatively, each compound being intermediate in phenotype between the two homozygotes from which it is derived. It is as though the different regions of the arista require different concentrations of some substance to make them tarsus-like, and the various allelomorphs each produced a definite quantity of this substance. Or one might suppose that the variation in effect is due to a variation in the time at which the "tarsus-substance" reaches a threshold concentration, this occurring so late in *ss^{a-B}* that most of the antennal disc is already determined and no longer capable of being affected.

Whatever the mechanism of the branching, there is no doubt that there are two alternative ways in which the antennal disc can develop. These alternatives are fairly sharply contrasted. In *ss^{a-B}* flies the whole arista is not intermediate between a normal arista and a tarsus-like one, as it would be if, for instance, the central stem was inflated and the branches reduced. Instead the basal part is transformed into a fairly completely tarsus-like structure, and there is only a narrow transition zone to the distal region which is definitely arista-like. It is clear that some kind of threshold phenomenon is involved.

It is interesting to study the genetic control of these two alternative developmental tracks². Aristopedia has been combined with genes affecting the tarsi; *dachs d*, four-jointed *ffj* and approximated *app* all cause a shortening of the tarsus and a reduction of

¹ Braun, 1939.

² Waddington, 1940 a, b.

the number of joints from five to four. In aristopedia flies, they shorten the arista and reduce the number of joints in it. Thus when the antennal disc follows the tarsus-like developmental track, it is affected by the tarsus-affecting genes. These genes then help to define this track, although they have no effect on the track leading to the development of a normal branched arista. Similarly, genes are known which affect the arista track but not the tarsus track. One such is thread *th*, which reduces or even abolishes the side branches of the arista. This has no apparent effect on a tarsus-like arista, but affects anything which is developing in an arista-like way, in particular the terminal arista-like portion of an *ss^{a-B}* arista. Again aristaless *al* reduces the size of a normal arista but has no effect on aristopedia.¹

The leg genes and thread and aristaless affect the developmental tracks after the tarsus-or-arista branching point. Other genes are known which seem to affect the developmental processes before this point is reached. Thus in eyeless-dominant *ey^D* flies the antennae are often reduplicated, and the same reduplication is found in aristopedia eyeless-dominant individuals. Here the genetic effect is one with low penetrance, i.e. is only manifested in a low percentage of flies, but it must be exerted very early in development. Eyeless-dominant also causes a characteristic swelling of the base of the tarsus and derangement of the hairs there. It produces the same result in aristopedia aristae, and this part of its effect is presumably exerted, on the tarsus track only, after the branching point. Another mutant, dachsous-38*k*, was recently found in an aristopedia stock. By itself it has a profound effect on nearly every part of the fly, shortening the legs, including the tarsi, reducing the antennae, curving the wings and interfering with their venation, and producing a more thickset body. It affects the antennae both when alone and when in combination with aristopedia. One can interpret its action in two ways; either it affects both the normal arista and the tarsus-like tracks, or it acts much earlier and shifts the whole track system including the branch point.

The system of tracks and their genetic control has been symbolised in Fig. 9, where we have neglected the quantitative differences between the different aristopedia allelomorphs, and regarded the arista-tarsus branch point as occurring at the same

¹ Braun, 1939.

time throughout the whole arista, instead of at different times, or at different concentrations, in different regions.

In the examples which have just been discussed, it seems that the sequences of gene reactions must be described in terms of branching tracks, and that the presence or absence of particular genes acts by determining which path shall be followed from a certain point of divergence. We have to remember that the course of each branch of the complex track is controlled, like the course

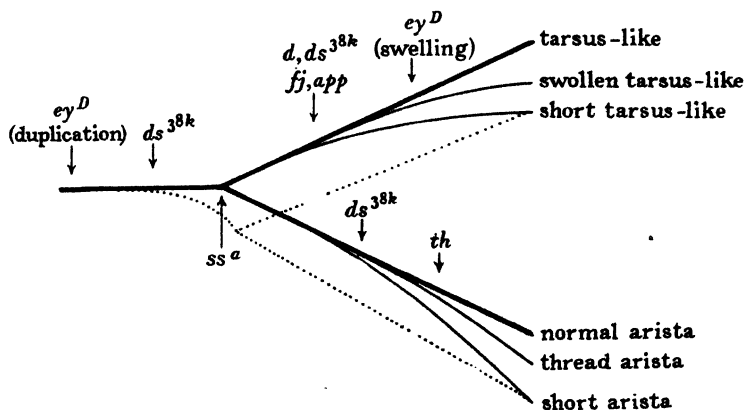


Fig. 9. The aristopedia developmental track system. The developmental process runs from left to right, either into the tarsus track above or the arista track below. Both these tracks are affected by various genes. The dotted lines represent one interpretation of the action of *ds^{38k}*, which affects both normal and tarsus-like antennae. Only the mutant genes have been inserted; their normal allelomorphs would act in the opposite sense.

of the simple curves we considered earlier, by the whole genotype or the greater part of it.

It seems then that the effect of a gene on a certain gene sequence may be confined to a certain period, when the process is actually at the branching where the gene is decisive. The actual time of the crucial period can be determined in some cases, e.g. by injection of lymph at different times in the *Drosophila* example discussed on p. 77. It is clear, however, that we can only discover in this way the period when the gene substances exert a crucial influence on the system which is actually developing into the eye pigment. The crucial period for the claret gene, for example, must be some time after the *ca⁺* substance is manufactured and poured out into

the lymph, and it is quite possible that, in the region where this substance is built up, development in claret flies deviates from that in normal flies at a much earlier stage. Several authors have suggested that the time of the sensitive periods for temperature changes which are found in some mutant types (p. 75) indicate the times at which the mutant genes concerned exert a crucial influence, but all we can really deduce is that reaction sequence set going by the mutant gene has a sensitive period at the time discovered, and we cannot, as Henke¹ has pointed out, assume that this sensitive period is the first period in which the gene is active and the mutant development deviates from normal development. The mere fact that limited sensitive periods occur, however, is evidence that the reaction sequences consist of successive reactions of different nature and sensitivity to environmental influences.

The genes we have just considered control the choice between two alternative modes of development which are quite sharply contrasted. As was made clear in the previous discussion, it is not intended to imply that all genes behave in this way. In discussing the embryological concept of competence, we saw that sharply distinct modes of development will be produced during evolution because definite and distinct tissues must be differentiated. An evolutionarily efficient genotype must therefore determine a system of developmental tracks which involve branching. It is to be expected that some gene substitutions in such a genotype will affect the choice of which alternative mode of development is followed; these are the genes we have just been considering. On the other hand, when a gene substitution is made in a normal genotype, the result is not necessarily, or even probably, well adapted from the point of view of evolution. Such a gene substitution will disrupt the carefully adjusted system of branchings; development, instead of following one of the normally possible tracks, will deviate into some quite abnormal path. For instance, whereas *ss^a* shunts the development of the antenna into a path, of leg-like development, which is part of the normal repertoire of a developing *Drosophila* (though usually played by another member of the cast), a gene such as *th* produces merely an abnormal, badly performed version of the antenna differentiation. *Aristopedia* has affected a choice between well-defined alternatives,

¹ Henke, 1937.

thread has blurred one of the alternatives on which the efficient carrying out of development depends.

As another example, consider the genes in *Drosophila* affecting bristles. Many of these cause the disappearance of certain of the normal bristles or the appearance of new ones. Presumably these genes act by causing certain cells which would normally produce bristles not to do so, or, in the latter case, causing normally non-bristle-producing cells to become so. They can be compared with ss^a , in that they bring it about that one of the normal types of differentiation is followed in an abnormal place. On the other hand, there are many other genes which merely reduce the size of the bristles. These again do not break up the normal types of differentiation, but affect quantitatively the amount of material which undergoes the bristle differentiation; they can be compared with the different allelomorphs of ss^a . Finally, there are genes such as forked, singed, etc. which cause the production of warped and bent bristles, and which therefore must be regarded as breaking down the normal type of differentiation. The first group of genes tends to shunt development into some particular path, either towards or away from bristle formation; the second group affects the quantity of material which is shunted; while the genes of the third group are part of the system which defines the "bristle developmental path"

One must think of the genetic control of a developmental path as a very detailed and continuous action on every phase of the developmental process. The organ which has been most fully studied from this point of view is the wing of *Drosophila*. Goldschmidt and Auerbach¹ have published observations on some phases of the development of a few mutant types, and I have recently made an investigation of the development of the normal wing and of 38 mutants. These 38 genes affect 16 different, but not necessarily independent, processes which occur during wing development. These processes may be listed, approximately in chronological order, as follows:

1. Before the wing is everted, it consists of a thickened region of the surface of the mesothoracic imaginal buds. Each bud is an ovoid sac, and the wing region is on the posterior part of the ventral surface; it will eventually be folded into the sac, and then pushes out through the dorsal surface as a flat plate, which is composed

¹ Goldschmidt, 1938; Auerbach, 1936.

of two epithelial layers folded together. Before this folding occurs, a pattern of longitudinal veins is determined on the future dorsal wing surface at some time during the late larval period. The process is influenced by *shifted-2*, which causes the veins to diverge from one another at a smaller angle than normal (Fig. 15). The genes *four-jointed*, *dachs* and *approximated*, besides their effects on the legs (p. 129), also cause an increase in the angle between the veins and probably act at this time.

2. The fold by which the wing region is pushed into the bud and thus everted eventually becomes the wing margin. Normally it coincides with a line which is determined to develop the marginal vein and bristles. But the position of the fold is affected by the "scalloping" genes (*scalloped*, *vestigial*, *vestigial-nicked*, *Beadex-7*, *Beadex-C*, *Beadex-3*, *Lyra*, *cut-6*, and *Xasta* (Fig. 12) were the ones studied). These shift the fold in relation to the determined margin, so that some of the margin is missing from the adult wing.

3. As soon as the wing region has folded together, the veins of the prepupal stage can be seen as hollow tubes between the two surfaces. Their development is affected by *cubitus interruptus* and *cubitus interruptus-Wallace*, which cause the disappearance of certain regions.

4. At the same time the wing expands, both by cell division and cell expansion. The relative rates of cell division in different directions are affected by *broad*, *expanded*, *lanceolate-2* and *narrow*, of which the first two cause the wing to be relatively broader and the last two relatively longer. The expansion of the cells is partly inhibited by *miniature* and *dusky*.

5. The base of the wing does not normally begin constricting so as to form the wing-insertion until some time later, but in *Wrinkled* flies the process begins at this time.

6. About 9 or 10 hours after puparium formation the wing becomes highly inflated by internal pressure, which forces the two surfaces apart. The height of this inflation corresponds with the beginning of the true pupal period, during which the wing contracts again to a flat plate. There are four main factors concerned in this contraction.

(a) The contractility of the wing epithelia. This is relatively increased in *dumpy*, *dumpy-02*, *Blade* (of *D. pseudoobscura*) and probably in *spade*. The resulting deformation may be a shortening of the wing, as in *dumpy* and *spade* and occasionally in *Blade*, or an elongation as is more usual in *Blade* (Fig. 15).

(b) The veins. Possibly different vein patterns have an effect on the contraction, but it is slight.

(c) The general shape of the wing. In scalloped wings with a generally elongated shape the elongation is increased, in those with a rounded shape the breadth is probably increased.

(d) The expressing out of the wing of the body-fluid and its contained cells. In *balloon* blood cells become caught among the processes which extend between the two epithelia. In *bloated* droplets of fluid remain within the cavity of the wing and become clothed with cells which should belong in the epithelia.

7. As the wing contracts and the two epithelia close together, the definitive veins appear as lacunae. The distal tips of these fail to persist in *veinlet*, and the middle portion of the third longitudinal vein (the medius) disappears in *tilt*; *abrupt*, which removes the end of the fifth vein, probably acts at this stage. The veins which were destroyed by *cubitus interruptus* do not appear at this stage.

8. The radius, or second vein, is formed in a different way to the other longitudinal veins, by the coalescence of small spaces. This process is inhibited by *radius incompletus* (of *D. simulans*).

9. The posterior cross-vein is formed as the last trace of the central cavity of the wing. This does not persist in *crossveinless*. The position of the cross-vein is not determined at the same time as that of the longitudinal veins, since it is dependent on the relations between the longitudinal veins in its neighbourhood, and these may be altered, by genes such as *veinlet*, for instance, as late as the pupal period.

10. After the wing has attained the form of a flat plate, the veins continue to become narrower. This is inhibited by *Delta-6*.

11. The intervein material develops as wing membrane. This is secondarily affected by *balloon*, since the surface of the cavities formed around entrapped blood cells develops abnormally thick chitin. It is also affected by *plexus*, *net* and *blistered-2*, which cause the persistence, sometimes followed by coalescence, of cavities within the intervein spaces, and thus lead to the deposition of chitin and the formation of extra veins.

12. The margin becomes thicker, particularly along the anterior edge. This process is increased unduly by *Delta-6* and *net*.

13. The formation of extra veins entails a greater contraction of these cells than would occur if they developed as membrane. This may cause the stretching of the remainder of the membrane, a phenomenon which is most noticeable in *net*.

14. The wing finally expands in area by enlargement of its cells. This process is to some extent inhibited by *miniature*, *dusky* and *bloated*.

15. After emergence from the pupa, the wing, which has become folded owing to the above-mentioned expansion, is unfolded and stretched by the pressure of the internal fluid. This is partially prevented in *Wrinkled* by the narrowness of the wing-insertion consequent on its precocious and exaggerated development.

16. The wing dries out to a thin flat plate. This is affected by *Curly*, which causes an undue contraction of the upper surface, and by *curved*, which has a similar effect on the lower surface.

This complicated series of events during wing development is probably typical of the conditions during the development of other organs, and the mutants which have just been described show the kind of genetic effects one must expect.

Genes which blur the developmental alternatives are certainly much the commoner type. The blurring very usually takes the form of a quantitative alteration from the normal. The genes involved, in fact, are very often hypomorphs, in Muller's sense. They do something similar to their normal allelomorphs, but do it less efficiently, and thus lower the slope of the time-effect curve of some developmental reaction. We can regard these genes, in fact, as being the members of the genotype which define the sides of the developmental valleys. The evocator genes, which shunt development along one or other of the possible developmental branches, may sometimes be neomorphs, i.e. they may do something quite different to the normal allelomorph. For instance, the effect of the *aristopedia* allelomorphs on the antenna may be one which the normal allelomorph does not in any way parallel. But this is by no means necessarily so; a choice of a different track may be a consequence of a threshold phenomenon, and thus fundamentally due to a purely quantitative variation, governed by a "quantity gene". Muller's classification, and the classification we could make here into what one might call evocator and competence genes, are based on disparate considerations, and have no simple relation to one another.

In all the examples we have so far considered, the effect of the genes has been more or less localised, in an eye, in a bristle, in an antenna and so on. This localisation is, of course, not a necessary

characteristic. An alteration in an early stage of a developmental sequence may later cause abnormalities of development in many different parts of the organism; it may produce, in fact, a syndrome of effects. Perhaps the best known examples of this are effects produced through the mediation of glands of internal secretion. The difference in structure between breeds of dogs, for example, are probably dependent on genetically controlled variations in hormone production. Similarly, hereditary dwarfism may be primarily due to a lack of pituitary secretion, which brings in its train a whole set of alterations affecting every part of the body.

Landauer¹ has devoted a series of papers, which he has recently summarised, to the frizzle fowl, which is a good example of such non-localised effects. Fowls of this genotype show not only the abnormality of the feathers from which the gene takes its name, but also an augmentation of the basal metabolism, hypertrophy of the heart, an increase in the rate of heart beat and of the volume of the blood and sometimes of the number of lymphocytes and immature red cells. The spleen is enlarged, and so are the crop, gizzard, pancreas and kidneys, while the adrenals and thyroids are abnormal and the gonads usually reduced in size. All these effects are produced by a single gene, which is partially dominant over its normal allelomorph. Landauer succeeded in showing that it is extremely probable that they all trace back to a single starting point, the inadequate keratinisation of the feathers, which causes them to be lost, the body to become bare and the rate of heat loss thus largely increased, calling for the adoption of all kinds of compensatory phenomena. Grüneberg² has discussed somewhat similar cases in the rat. He shows how a single original effect, an abnormality of the cartilage, produces a large number of secondary consequences; his diagram of the causal relations involved is reproduced in Fig. 10.

The production of numerous effects in different parts of an organism by a single gene has been referred to as pleiotropy. But clearly there is no great difference in principle between the production of many effects in different regions and the production of many effects in a single part. The alteration in the *artista* caused by *ss*^a could be analysed into numerous elementary effects, and we could, if we wished, speak of the "localised pleiotropy" of the gene *ss*^a. In both cases we are dealing with an initial change

¹ Landauer, 1937.

² Grüneberg, 1938; Fell and Grüneberg, 1939.

which gives rise to conditions under which a constellation of genes react with one another in abnormal ways. The correlated set of processes which causes a pleiotropy is not different in principle to the correlated set which is involved in the differentiation of an organ. Only in the first case, the pressure of natural selection will have ensured that the genotype is so equilibrated

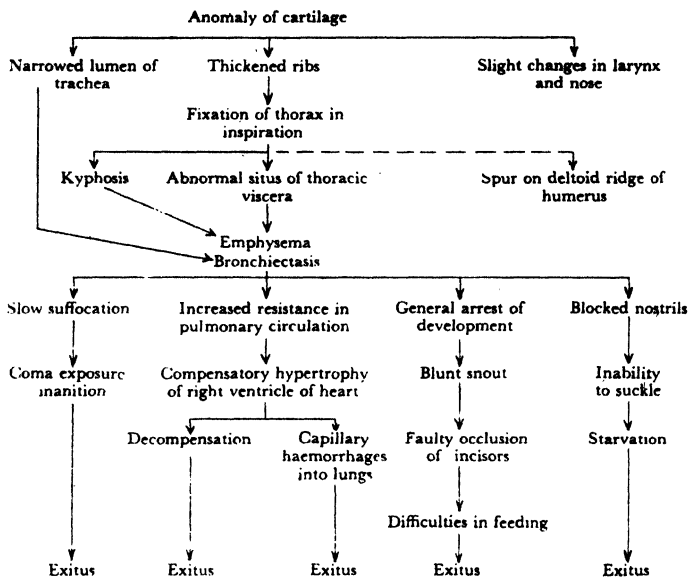


Fig. 10. Diagram of the probable causal relationships of the "pleiotropic" effects" of a lethal gene in the rat. (After Grüneberg.)

that the processes work harmoniously together to give a comparatively constant and invariable result, while the correlations involved in a deleterious pleiotropy will not have been subjected to the same selection and may be expected to be more loosely organised and to produce a more variable picture.

We may expect, however, to find some cases in which non-localised pleiotropies or syndromes have some of the definiteness which is characteristic of organ-formation. Goldschmidt has claimed that this is the case for sex differentiations. He supposes that development must be either male or female, but may, in intersexes, switch over from one to the other at some stage

during development. This may be compared with the development of the antenna in aristopedia; the arista, as we have seen, can develop either as a leg or an arista and can switch from one to other at an intermediate stage. The only difference is that the male-female alternatives affect many parts of the organism, and are thus syndromes in the ordinary sense, while the leg-arista alternatives apply only to the arista. But this is clearly not a matter of fundamental importance; in fact we might speak of any course of differentiation, which involves a co-ordinated system of changes, but which occurs in only one place, as a localised syndrome.

It must be pointed out that other authors¹ have disputed Goldschmidt's hypothesis as to sex differentiation, and supposed that the alternative between maleness and femaleness is not so definite as he suggested. They claim that an intersex, instead of undergoing development as one sex for part of the time and then switching over to that of the other sex, differentiates in an intermediate way throughout. If this is so, then the intersexual condition is no more a case of sharp alternatives than is pituitary dwarfism. It becomes merely one of the numerous cases in which the normal well-defined alternatives are disrupted by changes in the genotypic system on which they are based. It is clear, however, from our discussion that there is no reason why both sorts of intersexuality should not exist.

The degree of contrast between two alternative modes of development is affected by two conditions. In the first place, the two modes will tend to become the more obviously different from each other the longer the interval between the branching point and the attainment of the final adult form; and, secondly, sharp alternatives can only be formed if each of the modes is a resultant of many influences, whose interactions lead to an equilibrated condition which eliminates intermediates. These two conditions will not necessarily vary together. If a branching occurs late in development, the subsequent differentiation will be short in duration, but the system concerned may be complex enough for the delimitation of perfectly sharp alternatives.

The Epigenetic Landscape.

The similarity between the theoretical schemes we have arrived at on embryological and genetical grounds is immediately apparent. In embryonic development we are confronted with

¹ Baltzer, 1937; Bridges, 1939.

alternative modes of development, the choice between which is taken in reference either to an external stimulus, in inductive development, or to an internal one, in mosaic development. In considering the effects of genes, we find alternatives the choice between which may be taken in response to diffusible substances, as in the *Drosophila* eye colours, or apparently in response to internal factors as in aristopedia. It is clear that we have merely followed two different methods of approach to the same phenomena, and that the two theoretical schemes are in fact identical. The genetical approach has, however, added something to the picture drawn from embryological considerations. We had described competence as a state of disequilibrium in a complex system of reactants, and had suggested that the reactants are ultimately genes or gene-products. We have obtained definite evidence of this from the fact that the course of the alternative branches is under genetic control. Thus one of the reactants involved in the arista developmental track is the gene thread or its products. Moreover, we have seen that the existence of sharply contrasted alternatives, although it may be most clearly exposed by genes such as aristopedia, is primarily a property of the normal genotype, in which it is presumably ensured by natural selection; in many mutant types the sharpness of the alternatives is blurred.

The system of developmental paths has been symbolised in two dimensions as a set of branching lines. Perhaps a fuller picture would be given by a system of valleys diverging down an inclined plane. The inclined plane symbolises the tendency for a developing piece of tissue to move towards a more adult state. The sides of the valleys symbolise the fact that developmental tracks are, in some sense, equilibrium states. The meaning which must be attached to this term in such a context may at first sight not be obvious, since the developmental processes move along the tracks and do not stop anywhere in their course. It is not meant, however, that any one point on the track is a position of equilibrium; it is the track as a whole which, compared with any other line lying between the tracks, is a description of an equilibrium. The equilibrium is a moving one and the state of the system changes as time passes. But it is an equilibrium in the following two senses. Firstly, it is a definite, and normally repeatable, result of a whole complex of factors. Consider the developmental track of the antenna of *Drosophila*; it is affected by several genes which

we know of and certainly by many of which we know nothing. The reactions of these genes with one another (and with the environment) interlock so as to define a developmental track which will always be followed by the antennae of flies of a certain genotype. Secondly, the normal developmental track is one towards which a developing system tends to return after disturbance. This is another way of formulating the phenomenon of embryonic regulation.

Regulation only takes place during a certain period of time. During this period the developmental track can be represented as a valley with gently sloping sides; if the course of development is disturbed, the point representing its disturbed state will be somewhere off the track, and if it is still within the valley, the process will regulate by running down the side of the valley until it reaches the normal track at the valley bottom. After the period of regulation, the developmental track is still in a valley in the sense that it is the most probable sequence of events. But if the process is disturbed, there is no tendency for regulation; we must represent the valley as having vertical sides, and located in a plain.

This symbolic representation of developmental processes can be spoken of as the "epigenetic landscape". It would be difficult to find a similar configuration in any actual piece of country. As one goes downhill, the valley which was originally wide and gently sloping, branches into more and more subdivisions, some of which (representing tracks realised only under the influence of special genes or environmental conditions) may be hanging valleys whose floors disembouch up the side, above the main valley bottom. It is an amusing landscape to picture to oneself; and I think it expresses, formally at least, some characteristics of development which are not easy to grasp in any other way.

The arguments which have led to this picture have related entirely to the substantial side of development; we have considered the embryonic development of new types of tissue, by evocator-competence reactions, and the genetic control of particular kinds of substances. We must now turn to the other aspect of development, which we spoke of as individuation. We shall see that in many respects it presents similarities with the processes which have already been discussed, although there is a residue of morphological changes which we cannot as yet bring within the same framework.

CHAPTER VIII

INDIVIDUATION

Individuation and Evocation.

THE concept of individuation was derived from a consideration of regional determination. There are several lines of evidence which show that the regional characteristics of the organiser behave differently to the basic property of evocation which is common to all parts.¹

Firstly, the regional characteristics of an induced axis are strongly affected by the host embryo, while the mere occurrence of induction (evocation) is not usually influenced in this way. The influence of the host would seem to be only explicable if the processes determining regional properties are spreading and not strictly localised. It should be noted that this distinction between evocation and individuation sometimes breaks down, since in some situations, a host may so strongly affect the regional characteristics of a graft that the latter ceases to belong to the organiser (e.g. by being converted into lateral plate mesoderm in the chick) and then does not produce an induction.

Another set of facts relating to individuation also point to a difference between it and evocation. The phenomena of regional determination are connected with a tendency to form a complete unit. This is shown in two ways: by the regulation of fragments of the organiser to develop into, and to induce, a greater region than corresponds to their presumptive fate; and by the amalgamation of graft and induction, or even of graft, induction and host, into a single composite organ or embryo. In interpreting the latter phenomenon, we have to bear in mind the possible relevance of the competence, since it is *a priori* possible that competent ectoderm has a tendency to react to a purely evocatory stimulus by the formation of a complete organ or region of the neural tube (p. 50). Moreover, the assimilation of graft to induction, to give a composite organ, might be a secondary process, something to do with a mutual adjustment to each other occurring after the induction had been completed. But the assimilation of two organisers, as

¹ Waddington & Schmidt, 1933; Waddington & Needham, 1936.

when host and graft unite, and the regulation of isolated pieces of organiser, show that the unit formation is really a property of the regional aspects of the organiser and cannot be attributed wholly to the competence.

Lastly, we must consider the fact that the organiser has different properties in its different parts, while some sort of induction can be performed by homogeneous solutions, all of whose parts are equal. It is clear that a single solution does not possess all the properties of the living organiser; on the other hand it has something in common with the organiser, since both can induce. Evocation is the process for which the common factor is responsible, individuation the process which differs in different regions of the organiser. The common factor, since it exists in solution, is presumably a chemical substance, but at this point in the discussion we have no indication of the nature of the individuating factors. They might be different quantities or concentrations of the basic evocator substance, or they might be different substances specific for the various regions of the body, or some other differential conditions.

The fact that the dead organiser, and, still more, inducing solutions, cannot be supposed to share all the properties of normal living organisers, was pointed out in the very first communications announcing that inductions had been obtained after the organiser had been killed. Spemann¹ spoke of the dead organiser as producing merely a conversion of presumptive epidermis to neural tissue, but Waddington² showed that this is an undue simplification, since in his inductions in the chick induced notochord was found as well as induced neural tissue. But it was clear that in this case the regional characteristics of the induced axis were dictated by the host rather than by the dead material. The distinction which is necessary is not one between an inducer of several tissues and one of neural tissue alone, but between an organiser with regional properties and one without. The problem of whether evocation involves the production of an organ or of a mere tissue, and the connected question of whether there is a separate evocating substance for mesodermal induction, are not essentially involved in the distinction between individuation and evocation and must be left for further experimental investigation (cf. p. 103).

¹ Spemann, Bautzmann, Holtfreter & Mangold, 1932.

² Waddington, 1933 *a*.

The fact that dead organisers are not fully representative of living ones is admitted by most of the authors who have reviewed the subject. Thus Weiss¹ writes: "Waddington, noting the significant difference between the effects of living and dead 'organisers' in the chick embryo, is more cautious in his conclusions and clearly distinguishes between the 'evocating' activity of the inductor and the 'individuating' activity of the host field, all the organising, i.e. pattern-determining, effects going to the credit of the latter. It appears that this is the only stand which one can safely take in view of such facts as have come to our knowledge so far." While there is thus complete agreement on the necessity for some distinction between evocation and individuation, Weiss's attitude differs slightly from that which has been advanced here in that he does not consider the competence of the ectoderm as an entity separate from the individuation field of the host. The attribution of all the pattern effects to the host may turn out to be justified, but it cannot be assumed without question. We have seen that it is possible that evocation produces organs, not mere tissues; and if this is the case, some pattern effects, namely the production of a morphologically definite organ, while they would not be transmitted from the dead organiser, would also not be properly attributable to the host field; they would be a result of the competence of the ectoderm. All that must, in every event, be attributed to the host field, are the regional differences between different parts of the embryo.

Holtfreter² also appears to have thought that the distinction between evocation and individuation essentially involved attributing all pattern effects to the host field, and, since he had found some evidence of pattern effects when implants were made into isolated ectoderm, was inclined to reject the distinction. As we have just seen, his objection was based on a misunderstanding. Moreover, it is perhaps still not entirely clear that pattern effects are in fact produced when homogeneous evocators act on tissues removed from the field, since the implants he used were dead tissues, not extracts, and therefore not certainly homogeneous. This question has been discussed in another place (p. 50).

Woerdeman³ has also pointed out the difference between the action of living and dead organisers. He developed somewhat

¹ Weiss, 1935; 1939.

² Holtfreter, 1934 *b*.

³ Woerdemann, 1936.

further a train of thought which is also apparent in Weiss's account; he supposed that the dead organiser or evocator is a mere activator, which liberates the field which is lying latent in the reacting tissue. This point of view involves a thorough confusion of the ideas which we have distinguished as competence and the host's individuation field. It leads to difficulties of the following kind: the fields which are activated are only adequate to explain the facts of regional determination if they are closely connected with the host embryo; but if they are connected in this way, it is not easy to see why there should be any field capable of activation in isolated ectoderm, on which, however, it is well known that evocation is effective. If we assume the debatable fact that evocation can produce organs with a morphological pattern, it might be advantageous to speak of evocation as activating a field in the competent ectoderm, but there is no need to unite this notion with that of the individuation field of the host; the "competence field" would deal with the assumption of a definite form by the induced tissue, the individuation field with the adjustment of this form to the pattern of the host.

The Structure of the Egg.

In considering the different aspects of individuation, it is advisable to discuss first the processes which lead to the establishment of chemical differences between the various organs within the organiser; then to consider the chemical differences produced by induction; and finally to turn to the problems of the development of morphological patterns.

The first step in the establishment of local chemical differences within the amphibian egg takes place before fertilisation. At this time the egg has an animal-vegetative gradient, which is probably defined primarily by the increase in yolk content towards the vegetative end. This is not, however, the whole of the story, since in the first place the decrease is not uniform, there being a central comparatively yolk-free region which does not fit into the picture of a gradual transition, and secondly the position of the germinal vesicle near the animal pole cannot be neglected. The structure of the egg is certainly determined during its maturation in the ovary; probably the mode of attachment to the wall of the ovary, as well as gravity, play a part. It is to be regarded, not as a structure produced by the egg itself, but as one formed by the mother;

it is in fact a part of the maternal phenotype, just as is the structure of any other highly differentiated cell of her body. The well-known and classical case of *Limnea* provides a clear example of the effect of the maternal genes on the primitive structure of the developing egg.

It is somewhat uncertain how much more structure must be attributed to the unfertilised egg and thus laid to the charge of the maternal genes. It seems now to be generally agreed that a frog's egg contains a preformed plane of bilateral symmetry, which is, however, rather easily susceptible of modification.¹ Certainly in other forms, such as Ascidians, a plane of symmetry is present in a definitely determined condition. In the frog, the point of entry of the sperm is usually opposite to the position later occupied by the grey crescent, but experiments on parthenogenesis show that the position of the grey crescent is independent of the position of artificial stimuli, and it seems probable that it is the preformed plane of symmetry which determines the point of entry of the sperm rather than *vice versa*. It is likely, then, that in the unfertilised egg some precursor of the grey crescent, and thus of the organiser, is already in place.

What this precursor of the organiser is is a difficult question. Dalcq and Pasteels² have recently suggested that it is some property of the egg cortex, which is most intense in the presumptive dorsal lip and falls off in all directions from there. In their view the actual position of the blastopore, and thus of the definitive dorsal axis, is determined by the interaction between the yolk gradient and this cortical field; by altering the position of the yolk, for instance by inverting the egg and allowing the yolk to move under the influence of gravity, it is possible to shift the position of the blastopore without affecting the location of the cortical field. Their experiments, however, do not seem to have excluded the alternative hypothesis that the precursor of the organiser is an actual material substance which is itself shifted in all experiments in which the yolk gradient is moved. Since one can, at an early stage, clearly see that the grey crescent contains material different to that in the rest of the egg, it is probably advisable to consider the matter in terms of such material differences, at least until the existence of a cortical field can be conclusively demonstrated.

¹ Pasteels, 1937*b*.

² Dalcq & Pasteels, 1937; Dalcq, 1938.

If the plane of bilateral symmetry is fixed before fertilisation, the first organiser precursor must antedate the grey crescent, which does not appear till after fertilisation. About the material nature of the grey crescent we know little more than that it has a characteristic colour; about a precursor of the grey crescent, we have no certain information. The two main properties which we can associate with the grey crescent, or organiser, material at a later stage are certain metabolic peculiarities and the presence of the free evocator. We do not know, with any certainty, when these properties first appear. The metabolism of the organiser has been measured in pieces removed before they had completely invaginated, and in some cases before they had begun to invaginate. There is therefore no reason why the metabolic peculiarities of the organiser should not have arisen at a much earlier stage, perhaps even at the time of appearance of the grey crescent. Similarly the evidence as to when the evocator becomes free is scanty; the point of view developed earlier suggested that the liberation of the evocator is connected with the metabolism of the organiser, but if we confess to ignorance of when this metabolism first sets in, we have no grounds for an *a priori* guess as to when the evocator is liberated, (cf. p. 39).

Local Chemical Differences.

The localisation of the organiser is an example of individuation affecting the whole egg. Similar processes of localisation soon begin to occur on a smaller scale within certain regions of the egg. Holtfreter¹ has shown that in the early gastrula stage there is a fairly strong localisation of different organs within the endoderm. If small pieces are isolated, they differentiate to their presumptive fates. The various tissues are in so far determined that they have separate and distinct capacities for self-differentiation. At the same time, the determination is certainly very incomplete since it is well known that an organiser graft at this time can still induce the neighbouring endoderm to form the appropriate organs.

The phenomena concerned in such localisations have been most fully studied in connection with the mesoderm. Holtfreter's isolation experiments have brought to light a considerable power of self-differentiation into presumptive fate in all regions of the mesoderm ring. Isolates from the ventral region, in fact, scarcely

¹ Holtfreter, 1938a.

deviate from the normal course of their development; but the same region can fairly easily be assimilated to an organiser graft and caused to develop into other mesodermal structures. On the other hand, the dorsal material is not so easily affected in its histogenesis by grafting, since grafts of it when placed in more ventral regions of the mesoderm usually continue their normal development into chorda, etc.¹ When isolated, however, the dorsal material shows that it also is not by any means fully determined, since fragments whose presumptive fate is notochord may in addition form muscle, neural tissue, epidermis, etc. The dorsal material, in fact, combines a strong tendency to self-differentiation with an equally strong tendency to form other tissues by regulation when it is isolated; the ventral material lacks the second of these capacities. In both regions, the determination of the different tissues gradually increases during gastrulation and at the end of gastrulation is becoming complete even in the ventral material in which the change proceeds most slowly.²

The different parts of the dorsal region, in spite of their tendencies towards regulation, have certain other labile fixed characteristics, namely tendencies towards regional specificity. The presumptive anterior material tends to develop head structures, the presumptive posterior material trunk or tail structures. These potencies can, in the early stages, be altered by appropriate grafting.³ If small pieces of presumptive anterior and posterior organiser are exchanged, or if a central strip of the organiser is reversed, a normal embryo may develop, showing that the grafts have developed in accordance with the region in which they find themselves rather than that from which they came. By the end of gastrulation, this lability has been lost, and pieces of posterior organiser, when substituted for the anterior organiser of a young gastrula, still develop into their presumptive fate.⁴

Rather similar conditions probably exist in the chick. In very extensive work the American authors⁵ have described the differentiation of fragments of the blastoderm when transferred to the chorio-allantoic membrane. The fragments usually contained mesoderm as well as ectoderm (and often endoderm as well), and some of the differentiations obtained must have been due to in-

¹ Bautzmann, 1933; Töndury, 1937.

² Yamada, 1937.

³ Waddington (unpubl.); cf. Abercrombie & Waddington, 1937, for the chick.

⁴ Hall, 1937; Lopaschov, 1935 b.

⁵ Cf. Rawles, 1936; Rudnick, 1935.

duction. There is considerable evidence that the conditions for development are not in all respects satisfactory, but even so one obtains a picture not only of an increasing capacity to differentiate into presumptive fate, but also of considerable regulation to give more than presumptive fate. The incompleteness of our maps of the chick blastoderm, and the difficulty of isolating clean tissue fragments, renders the chick not a very suitable object for work of this type. Beyond noticing the general similarity of the conditions to those found in the Amphibia, there is little to be gained from discussing the results in more detail.

We have then a picture of a gradually increasing specificity of the various regions. The specifications are certainly primarily chemical in nature. The mode of their origin is not definitely known, and several suggestions are possible. The fact that change in regional character can at first be easily brought about indicates that in early stages the potencies for several different kinds of development are simultaneously present; the gradually increasing specificity shows that one of these is preferred and allowed to proceed. Perhaps the easiest picture to make of this is to envisage the various specific kinds of tissue as represented by autocatalytic substances competing for the same substrate. Then quite a small initial advantage will in time lead to an enormous preponderance of the favoured substance if the situation is allowed to develop unchecked. The equations which would be required to formulate the matter would be very similar to those investigated by Lotka, Volterra and others who have dealt with the problem of a population of two predators competing for the same prey. It is interesting to note that in the population cases it has been found that some of the systems of equations have periodic solutions.¹ It is not impossible that a similar situation may hold here, and that the interactions of two autocatalytic substances may explain the frequent periodic phenomena of development, such as the formation of somites and other segmental organs.

The alterations which are brought about by grafting, and the regulation which follows isolation, show that the substances concerned in the localisation of the different organs must be diffusible. The diffusion must be possible both into the external medium, as in isolation experiments, and from place to place within the tissue. In Amphibia one gets the impression that

¹ Cf. Kostitzin, 1937.

diffusion from cell to cell within a tissue is somewhat easier or at least more uniform than diffusion from a cut edge into the medium, since regulation is usually more extensive, and nearly always leads to a more regular and harmonious result in grafts than in isolates.

Regional Induction.

The diffusion of organ-specific substances is also attested by the facts of regional determination by an inductive mechanism. The fullest investigation of regional induction is concerned with the neural plate. Here we may conclude that the different regions of the plate (brain, eye, trunk, etc.) are chemically different from one another at a fairly early stage, since quite soon after their formation they can be shown to possess different inducing properties when they function as secondary organisers, the eye for the lens, the forebrain for the nasal placode, etc.¹ There is also considerable evidence for the existence of chemical differences in the inducing substratum, the roof of the primitive gut. In the first place, the fact that the mesoderm as a whole is certainly differentiated into chemically distinct parts, with specific tendencies to form particular tissues such as blood, nephros, heart, somites, etc., makes it easy to accept a similar chemical differentiation of the gut roof into a brain-inducing region, a trunk-inducing region and so on. Moreover, Lehmann² was able to show that the different regions of the organiser have specific periods in which they are particularly sensitive to the effects of lithium solutions. Thus the entomesoderm which underlies the most anterior part of the head is most sensitive at about the middle of gastrulation, while posterior to this there is a gradual transition from the posterior head organiser, which is highly sensitive in early gastrulation, backwards to the tail organiser, which is most sensitive in the early neural plate stage. It seems not impossible, from Lehmann's account, that for everything except the most anterior head organiser, the period of maximum sensitivity falls at or immediately before the actual time of invagination, and in this case the temporal sequence of sensitivity may indicate, not material differences but only differences in the time of attaining the same condition. But even if this is so, it appears that at least the most anterior material has a particular character which distinguishes it from the remainder.

¹ Cf. Woedermann, 1938.

² Lehmann, 1936*a*, 1938.

The chemical differences between the regions might, on the one hand, be due to different concentrations of the same basic substance. On the other hand, it is equally possible that each region contains a specific substance which is confined to it and which is the cause of its peculiar properties. These specific inducing substances would have to be additional to the basic evocator, which is the substance active in non-specific extracts of the whole organiser. They may be referred to as modulators,¹ since their function is to modulate a merely neural induction into a specific kind of neural tissue characteristic of a definite region of the neural tube.

The experimental data are perhaps as yet scarcely adequate to exclude either of these hypotheses, but as far as they go they seem to favour the existence of specific modulators rather than mere variation in concentration. As to the latter, we can only say that there is no evidence that they exist, at any rate along the length of the embryonic axis, or that they play any causal role in determining regional specificity. No evidence of regional effects has been seen in experiments with chemical evocators, in which many different concentrations have been used. On the other hand, there is some, even if not entirely satisfactory, evidence of the existence of specific modulators. Thus Lopaschov² implanted dead eyes into young gastrulae and into isolated flaps of ectoderm, and observed the induction, not of general neural tissue, but specifically of eyes. A similar result was obtained by van Cleave.³ In certain experiments of my own (unpublished), the specific induction of eyes was found only when the implants were taken from fairly old tail-bud stages; dead brain material from open neural plate stages did not induce any specific structures, but the experiments are not yet numerous enough for this negative result to have much weight.

The existence of specific modulator substances is also suggested by the important experiments of Chuang,⁴ who inserted fragments of adult newt liver and mouse kidney into isolated flaps of ectoderm. As we have seen (p. 51), fairly well-formed organs were induced. When liver was used as an implant, there was no marked preponderance of structures characteristic of any particular region of the body, but the kidney implants were found to induce only head organs and never (in explants) organs such as chorda, muscle

¹ Waddington, 1938*b*.

² Lopaschov, 1936.

³ Van Cleave, 1938.

⁴ Chuang, 1938. Cf. Toivonen, 1938*b*.

or tail which belong to more posterior regions. These trunk organs all include mesodermal structures, and it is possible that the failure of kidney as opposed to liver to induce them is partly due to the absence of a specific evocator for mesoderm. But it is difficult to believe that a mere absence of mesoderm causes all the organs induced by the kidney to be head organs; it is easier to understand the phenomena if we may postulate the presence in the kidney of a definite "head modulator" which causes the induced neural tissue to be brain tissue.

The process of regional specification has two parts: the arising of chemical differences in different regions of the organiser, and the transmission of regional character by induction. For the first of these our best model is the process by which the organiser itself becomes localised within the egg; there is no reason why the smaller-scale differences within the organiser should not also be originally metabolic, and only later become transformed into differences depending on the presence or absence of particular substances. The process of transmission of regional character may, in its turn, be provisionally considered in terms of the diffusion of chemical modulator substances, and would then be analogous to evocation. There is therefore no need, in considering the chemical aspects of individuation, to invoke any new principles other than those which can be studied in the investigations on evocation. In fact, we may say that evocation of the neural tube is distinguished merely by the fact that it is the only one of these processes of chemical transmission which can be as it were isolated from the phenomenon of regulation. But as it becomes possible to induce, by chemical means, specific regions of the neural system, such as eye, brain, etc., these inducing reactions are themselves drawn out of the hotch-potch of individuation and become open to exact study.

The ease with which neural evocation can be rendered free from regulation probably depends on nothing more fundamental than time. At the beginning of gastrulation, the position of the free evocator is at least roughly fixed, and the evocator will diffuse out of this region more or less regardless of what the surroundings are. The positions of the modulator substances specific for the various regions of the neural system are, on the other hand, not yet fixed, probably because the substances are still in process of formation, and regulation is therefore still possible.

The situation therefore stands as follows. A living organiser possesses an individuation field, which (1) controls the morphological arrangement of the parts which develop from the organiser; and (2) is responsible for the tendency of parts of the organiser to regulate to a whole and to incorporate other suitable tissue in their neighbourhood. Such an organiser can transmit to neighbouring ectoderm (1) an evocator substance which calls forth neural differentiation; (2) perhaps modulator substances which specify the kind, and thus, to some extent, the regionality of the neural tissue; (3) an individuating effect which, by an assimilative mechanism, is responsible for the exact regionality of the induced organ. The competent ectoderm, in forming the induced organ will (1) react to the evocator (and modulators if such exist) by differentiating into definite kinds of tissue; (2) may perhaps in the absence of a living organiser develop into an organ the definiteness of whose morphology is ensured by an individuation field produced within the ectoderm itself; (3) but will, in the presence of a living organiser, develop into an organ whose morphology is profoundly affected by the individuation field of that organiser.

There is thus every reason to hope that future advances in knowledge will reduce the importance of the concept of individuation in connection with chemical differentiation. The formation of most distinct organs and tissues will probably turn out to be by evocation or some modification of it. That will leave under the heading of individuation only the processes of localisation of the different evocators and modulators. The investigations which are already under way on the localisation of the free evocator and the reaction between the evocator and competent ectoderm may be found to be an adequate model for the whole of the chemical aspects of differentiation. We shall still, however, be left with the problem of the assumption of definite morphological shapes, and to this we must now turn.

CHAPTER IX

MORPHOGENETIC MOVEMENTS

So far we have only discussed the chemical individuality of organs. But perhaps an even more striking characteristic of an organ is its morphology. Most organs have a shape, although an exception would be made by the blood, if one wished to consider it as an organ. In all animals above a certain small size life does not seem to be possible on a basis merely of local chemical differences, and organs with structures adapted for particular types of function are developed. It is this assumption of geometrical form which I wish to discuss, in certain of its aspects, in this section.

The morphology of an organ is not constant. At any given time, of course, and indeed throughout most of adult life, the organ consists of certain tissues occupying definite relative positions in space. But during development the pattern of an organ changes. It is almost certainly a mistake to attempt to abstract the other aspects of morphology away from this process of change. Merely to draw the structure of an organ at a definite stage and to discuss the static equilibrium which it represents is a very inadequate approach. The elements into which we should attempt to analyse the pattern of a developing organ are movements.

The morphogenetic movements about which we have most information are those of gastrulation. At this time the whole of the egg is still effectively a single pattern. The movements by which changes in this pattern are brought about, in the Amphibia,¹ can be analysed into the following constituents. On the dorsal side there is a powerful elongation in the direction of the anterior-posterior axis. This affects both the presumptive axial mesoderm and presumptive neural plate. The elongation is in both tissues accompanied by a narrowing from side to side; presumably this is brought about by an interdigitation of the cells, since they do not seem to change their shape markedly during the process. The elongation and narrowing is so considerable that the mesoderm and neural plate, which originally lay side by side in the same sheet,

¹ Vogt, 1929.

could not be accommodated within the embryo if they continued to do so. Normally the excessive length of the two tissues is taken up by the folding of the mesoderm round the lip of the blastopore so as to lie underneath the neural plate, but this folding can easily be disturbed, for instance by an alteration in the salt content of the medium, which leads to a failure of the elongating mesoderm to invaginate and thus to the formation of an exogastrula.

Correlated with this dorsal stretching in length and contraction in width there is on the ventral side a more or less opposite movement which primarily characterises the presumptive epidermis. This tissue expands in area, and at the same time the originally many-layered tissue becomes thinned down to a two-layered epidermis. The expansion in area is not uniform in all directions. Although there must be some extension in the direction of the animal-vegetative axis, so as to cover the disappearing endoderm, the main expansion must be at right angles to this, so as to compensate for the narrowing of the mesoderm and neural plate; and this lateral expansion of the epidermis continues while the neural plate folds up into a tube and eventually disappears from the surface.

A similar extension, predominantly lateral but also antero-posterior, must also affect the ventral mesoderm. But here it is combined with an infolding round the blastopore lip.

The movements of the endoderm are not so definite. It retreats within the interior of the embryo. This has sometimes been attributed entirely to the effects of the adjacent tissues, which are supposed to push the endoderm passively along. But Schechtmann¹ has recently shown that there is a movement of material from the vegetative pole towards the centre of the egg in the early blastula stage which cannot be attributed to any outside influence. Moreover, the formation of a dorsal trough, forming the floor of the primitive gut, would appear to be an autonomous production of the endoderm rather than something imposed upon it.

We know, thanks largely to Vogt, a considerable amount about how these movements fit together to produce the harmonious series of changes comprised in gastrulation. As to the mechanisms of the movements, and the existence of any causal connections between them, we are much less informed. If, provisionally neglecting the doubts raised above, we regard the movement of

¹ Schechtman, 1934, 1935

the endoderm as a passive response to the pushings of other tissues, we are left with the stretchings and bendings of the animal hemisphere to account for. As to the elongations, we have seen that the stretching of the animal material takes place predominantly in two directions at right angles at opposite sides of the egg; on the dorsal side the elongation is in the direction of the antero-posterior axis, while on the ventral side it is predominantly at right angles to this, although there is a considerable antero-posterior component due to the fact that it is the ventral material which has to cover up the endoderm. This relation of the two stretchings is perhaps dependent on nothing more than simple geometrical considerations. If a sphere is to be covered with elongated strips which do not easily bend, one of the natural arrangements is for the strips to be at right angles on opposite sides, as one can see by placing one's hands together to enclose a space, when the fingers correspond to the strips. Thus a strong movement of elongation in the antero-posterior direction on the dorsal side is bound to produce an expansion in the direction at right angles on the ventral side, independently of what the mechanism of the expansion may be.

We have in fact no information about the mechanism of these stretchings and expansions. We have very little idea even of the physical properties of the tissues concerned, their viscosity, tensile strength, etc., or of the order of magnitude of the work done or the forces produced during the movements. I have recently attempted to measure some at least of these magnitudes.¹ Small steel balls were placed in young newt gastrulae and subjected to magnetic forces produced by one pole of a long bar magnet. It was found that the force required to pull a ball clean through the roof of the blastocoel was about 7.2 mgms. per sq. mm. of the hemispherical surface in contact with the roof. The balls used were too large to slide between the cells, which were torn apart as the ball escaped. This measurement gives an indication of the breaking strain of the tissue. Of more interest is a series of experiments designed to measure the force which gastrulating tissue can exert. Smaller steel balls were placed among gastrulating endoderm or mesoderm cells, and the egg arranged in such a relation to the magnet that the applied force tended to check the gastrulation of the balls. It was found that both tissues could

¹ Waddington, 1939 *b*.

move the balls against a maximum force of about 0.3 mgm. per sq. mm. The fact that this is much smaller than the breaking strain is probably to be explained by the lack of rigidity of the tissue; when a ball was arrested by the magnetic force, the tissue probably flowed round it. These data, of course, represent only the beginning of an analysis of the problem, which one must hope will receive much more attention from biophysicists in the future.

The comparative independence of the movements performed by a piece of tissue when it is grafted into an abnormal situation strongly suggests that the cause of the movements is something inherent in the individual cells. It is not a characteristic of the egg as a whole, since in that case one would not, as one actually does, find that grafted presumptive mesoderm stretches and invaginates, grafted epidermis expands in area, etc. On the other hand, the cells as such seem to play little part in the movements; it is certain that the elongation of the tissue is not accompanied by an equivalent elongation of the individual cells. It is as though the cells were swept along in the stream of elongating mesoderm and neural tissue like corks floating on a river. The causative agent of the movements must apparently be something at once very small-scaled and transgressive of cell boundaries. Perhaps one could imagine some sort of intimate structure of the protoplasm, such as a fibrillation which was not stopped by the cell membranes.

The idea that gastrulation movements may be due to phenomena of this kind, akin to the formation of liquid crystals, is also suggested by the interesting analogy between the geometry of the gastrula and that of the lens. The structure of the latter,¹ in fact, is extremely interesting on its own account, apart from whether it is taken to give useful hints as to the interpretation of gastrulation. Lenses of a simple type, such as those of the *Selachia*, are approximately spherical bodies, which are built up as a series of concentric shells. Each shell consists of a layer of fibres. These fibres run between the proximal and distal faces of the lens, but on these faces they converge not to points but to lines. The line on the proximal face is usually horizontal, that on the distal face vertical. Here again, then, we have a structure consisting of a spherical shell on which are marked out two lines at right angles on opposite sides. The particular interest of the lens is that

¹ Cf. Mangold, 1931.

here it is quite clear that the two lines represent one solution of the geometrical problem of constructing a spherical shell with fibres. Is it possible that the movements in the animal half of a newt gastrula are caused by the formation of fibres? If one imagined that an originally non-fibrous material became condensed into fibres, more markedly on the dorsal than the ventral side, the two directions of elongation, at right angles to one another and more definite on the dorsal side, would find an easy explanation. Naturally, any such fibres would have to be sub-microscopic in dimensions, since no trace of them has yet been seen. But it is well known that processes of fibrillation do occur in protoplasmic materials, presumably as a change in the protein constituents. A good example is the formation of the mitotic spindle, in which, moreover, it is known that the fibres visible in fixed preparations do not exist as such in healthy cells, but result from an invisible sub-microscopic fibrous structure which can only be detected by polarised light. Unlike the fibres of the spindle, however, those postulated to explain gastrulation would have to keep their relative arrangement on a scale larger than that of the cells. But it is a general characteristic of any theory of gastrulation movements that the causative agent must extend across cell boundaries, since the division of the mass of tissue into cells seems to have very little relevance to the morphogenetic movements. Harrison¹ has already suggested cell-transcendent arrangements of fibres to account for the polarities observed in transplanted limb-buds, and Needham² has drawn attention to the possibility that such phenomena may play an important part in morphogenesis. As we shall see (p. 111), a fibrillation can be proved to occur within some of the cells of the amphibian gastrula. The possibility that the dorsal elongation may be caused in this way is thus not so remote.

The mechanism of the inturning of the mesoderm is extremely obscure. It seems likely to depend essentially on a single initial step; if the stream of elongating presumptive mesoderm is once coaxed inwards, it will continue to roll round the blastopore lip. In sections of the earliest stage of gastrulation, it is apparent that the process starts in the large endoderm cells which lie slightly below the thinner marginal zone. When the invagination groove first appears, the cells which line it elongate in a direction at

¹ Harrison, 1936.

² Needham, 1936.

right angles to the surface, and at the same time their external surfaces decrease in area so that a depression is formed which is the origin of the groove. The process can easily be observed in action. If a very young blastoporal groove is cut out of the egg, it has at first a more or less smooth outer surface, and an inner surface made up of the bases of the elongated cells, which stick out rather like a bunch of turnips. In Holtfreter solution, however, the smooth surface almost immediately acquires a very strong curvature; in fact, it rounds up into a narrow cylinder and disappears from external view, being entirely covered by the bunch of elongated cells.

The lengthening of the cells might be a mere secondary consequence of this decrease in external surface, though it seems probably too marked to be easily accounted for in this way. It is, moreover, at least partly a result of the fibrillation of the cell proteins, but in this case the fibre axis, instead of lying in the tangential plane, as was suggested to account for the elongation of the presumptive mesoderm, is perpendicular to the surface. The occurrence of the fibres can in this case be definitely demonstrated by observations with polarised light. The cells lining the early blastoporal groove, like all those of the amphibian egg, are heavily laden with yolk granules which obscure the birefringence of the cytoplasm. But in these cells, alone of all those in the gastrula, there are yolk-free regions, namely the narrow ends which actually reach down to the blastoporal groove. These processes show, in the living cells, a weak birefringence which indicates the presence of fibres running along the length of the processes, that is to say, perpendicular to the outer surface of the egg.¹

The decision whether the invaginating material is to be directed inwards, as it normally is, or outwards to give exogastrulation, would seem to depend on whether the area of the external cell walls decreases or increases. This will depend on the relative surface tensions of the membranes in contact with the external medium or with other cells. It is therefore not surprising, as Holtfreter² in particular has shown, that comparatively slight changes in the salt concentration of the external medium can cause the normal gastrulation process to become converted into exogastrulation.

The processes which determine normal gastrulation inwards seem to increase in intensity as gastrulation proceeds, since in the

¹ Picken & Waddington (unpubl.)

² Holtfreter, 1933c.

weaker grades of exogastrulation it is always the anterior mesoderm which is left outside, while later the material is passed inwards in the normal way.

The foregoing account of gastrulation in terms of fibrillation processes is still for the most part no more than a hypothesis, and it is possible that other no less plausible suggestions could be made. Until we have much further information on the subject, some guide to experimental work, however insecurely based, is better than none. It is of the greatest importance for an understanding of development that we should be able to substitute for the biological concepts of cell streams, expansions, epiboly and such like some more definite physical notions which can account for the forces which must be exerted if the developing tissue is to change its shape.

Instead of adopting the inductive approach to the nature of these moulding forces we can consider them deductively. Rashevsky¹ has pointed out that metabolising systems such as cells set up a diffusion field, both within and around themselves. Any bodies within a diffusion field are acted on by resultant forces due to the unequal bombardment of their surfaces by the solute molecules. Rashevsky was able to show that cells in which anabolic metabolism is more rapid than katabolic will in this way tend to attract other cells, while if the metabolic flow is directed away from the cell, it will tend to repel others. If one may invoke attractive and repulsive forces between cells, many of the changes of shape during embryonic development can be accounted for. These investigations, however, are still in their infancy. It is of the greatest importance to discover what types of force are available from general considerations such as the occurrence of metabolism and diffusion gradients, but the importance of such enquiries must not obscure the fact (or, for that matter, be obscured by it) that it may be very difficult to apply the results immediately in practice. For example, before we can attribute changes in embryonic form to diffusion gradients, it is necessary to ascertain whether the latter produce forces strong enough for the tasks assigned to them. Moreover, our knowledge of the metabolism of different regions of the embryo is too slight for it to be possible to correlate, with any certainty, the postulated attractive and repulsive forces with the metabolic processes which are necessary to cause them. In

¹ Rashevsky, 1938.

some cases, it appears that the type of force which would have to be postulated to explain the changes in shape is actually not the kind which would be expected on the basis of what we yet know of the metabolism. For instance, the organiser region in the amphibian embryo behaves as though the cells attracted one another; one may remember the dorsal convergence of the mesoderm as well as the drawing down of the overlying ectoderm to form the floor of the neural plate. But the organiser is a region of high mitotic and glycolytic activity, which according to Rashevsky's account should lead to a preponderance of repulsive forces.

One fundamental characteristic of many developmental patterns which we have not yet discussed sufficiently is the capacity of a mass of tissue to regulate its shape after disturbance. If, for instance, a young amphibian gastrula is cut along the frontal plane, the dorsal half gastrulates in a manner proper to its reduced size, and produces a normal though half-sized embryo.¹ The extent of the invagination is adjusted to the amount of material available; and a similar adjustment occurs if the available material is increased by grafting extra ectoderm into a gastrula.² Regulation is, however, not by any means always complete. In the isolated ventral half of a gastrula a series of movements are carried out which are not very much altered from those which the same tissues would have performed if left in place in a whole embryo. An understanding of the processes involved is more likely to come from these cases of incomplete regulation than from those in which the movements can be perfectly adjusted. The character of the incomplete patterns may indicate some at least of the operative causes. But until we have a fuller understanding of the forces causing morphogenetic movements, it is not possible to discuss their modifications during regulation.

The gastrulation movements which were discussed above served mainly to provide examples of the kinds of elementary processes, such as fibrillation, changes in relative tensions of surfaces, etc., which can be postulated as causing the developmental movements. To give a true picture of the state of affairs in the embryo, we also require an example of the complexity of the interrelations which can be discovered even when a morphogenetic process is investigated purely in terms of biological concepts such as induction. As an example we may consider

¹ Spemann, 1903.

² Waddington, 1938c.

the folding of the neural plate into a tube, since here the complexity, although somewhat greater than in the gastrula, is not so great as to preclude useful discussion.

The first question which arises is the determination of the shape of the neural plate while it is still part of the surface of the spherical egg. Holtfreter¹ has shown that when pieces of ectoderm from a species with a small egg are grafted into gastrulae of a large kind, the size of the neural plate is conformable with the underlying host mesoderm. It is therefore natural to ask whether the size and shape of the plate can be determined entirely by the distribution of the evocator in the underlying mesoderm. We have reason to suppose that the evocator is set free at the blastopore, and it presumably diffuses laterally from the dorsal midline as the mesoderm moves forwards after invagination. The ectoderm moving towards the blastopore meets and overrides this stream of evocating mesoderm, so that it is the posterior end of the future neural plate which is first acted on by evocating tissue, while the anterior end is reached only later. The evocating tissue is widest at its anterior end, and gradually grows wider, by the lateral diffusion of the evocator, as invagination proceeds. One might make the hypothesis that the shape of the neural plate is a result of the combination of the ectoderm stream towards the blastopore, the mesoderm stream away from it, and the lateral diffusion of the evocator; the width of the neural tube at any level could not be greater than the width of the widest part of the evocating tissue at the time when the ectoderm passed over it. For instance, perhaps the most peculiar feature of the neural plate is the fact that it is wider near the anterior than it is farther back, and that the sides are curved so that of two points lying in the same line of the gastrulation movements, the anterior one may be in the plate while the posterior one is lateral to it. This could be easily explained if we supposed that at the time the determination was complete the lateral diffusion of the evocator had reached a certain distance from the midline at the anterior, where the mesoderm had been longest invaginated, but had not moved so far further posteriorly, where invagination had occurred more recently. (Fig. 11.)

In developing such a hypothesis there are several variables which would need experimental determination. The relative

¹ Holtfreter, 1935 *a, b*.

speeds of the tissue streams and of the lateral diffusion of the evocator would influence the final shape; so would any continuous production of the evocator within the mesoderm after it had been invaginated. Until these have been accurately

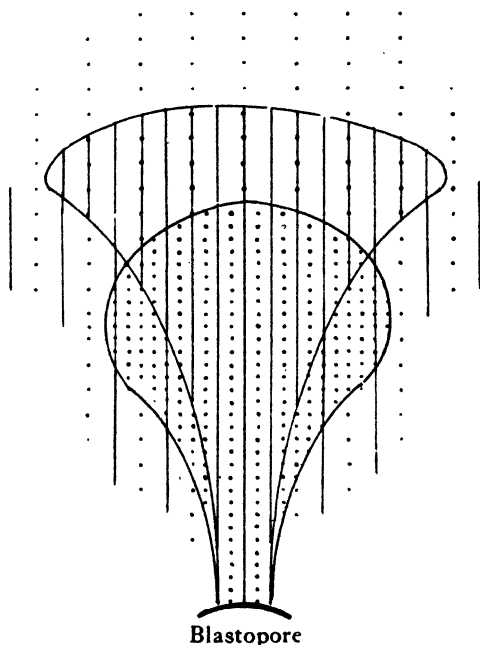


Fig. 11. The shape of the neural plate. The ectoderm, dotted, is moving down towards the blastopore. At the dorsal lip, the evocator is liberated, and as the mesoderm (lined) moves forward under the ectoderm, the evocator diffuses away laterally. After some time the area in which the evocator is present in the minimum effective concentration would have the shape of the closely lined region. At this time the ectoderm which had been underlain by evocating material for long enough to become determined as neural tissue would have the shape of the closely dotted region, which is similar to the shape of the neural plate.

studied, we cannot say that the hypothesis is fully adequate to account for the shape assumed by the plate. But there is some evidence that something of the kind may actually be involved. Waddington¹ has shown that the lateral mesoderm of a fairly late

¹ Waddington, 1936c.

gastrula has some inducing power when transplanted beneath young gastrula ectoderm, but its inducing power seems to be less than that of the dorsal material, since dorsal mesoderm will induce in the lateral ectoderm of a late gastrula where lateral mesoderm will not. It seems then that there is a falling off in evocating power from the dorsal midline towards the sides. This is perhaps combined with a falling off in the competence of the ectoderm. It may be that these two are causally connected, since we have seen that competence is lost more slowly in isolated ectoderm than in ectoderm in place in the lateral and ventral regions of the embryo, which suggests that the loss of competence is favoured by low concentrations of the evocator.

CHAPTER X

ORGANISERS AND GROWTH

The Determination of Relative Growth.

THE subject of growth is, of course, a very large and complicated one. Here we shall only discuss those aspects of it in which it comes in contact with the organiser phenomena.

We may first enquire what is the effect of evocation of the growth rate of the ectoderm. Casual examination of the embryo would suggest that the neural tissue of the tail-bud stage grows more rapidly than the epidermis, and this can be confirmed by counts of the frequency of mitosis in the two tissues. The data in Table 1, for which I am indebted to Mrs R. I. Schukoff, show that a similarly increased growth rate is also found in the neural tissue induced by various chemical substances. Clearly the growth rate must be considered as a histological character of the tissue, which is determined at the same time as the shape and arrangement of its cells. There is, however, evidence that differentiation and growth rate are not at all indissolubly connected. In fact, under abnormal conditions, such as those of tissue culture or chorio-allantoic grafts, there may even be some antagonism between them; slow-growing cultures often show better differentiation than faster growing ones, though too great a growth inhibition may of course lead to faulty differentiation. The characteristic of a tissue, in fact, is not so much an absolute growth rate, but rather a potential growth rate, which may be modified by external conditions.

Until we have some further picture of what occurs during carcinogenesis and evocation, it is probably useless to attempt to discuss the significance of the fact that the strongest chemical evocators yet discovered are also carcinogens or oestrogens. It certainly seems that the different actions of this whole group of biologically active substances must be in some way related, but as yet we can do no more than point out that most of their actions involve changing the growth rate and type of differentiation of the cells on which they act.

Table 1. *Mitotic rate in normal and induced tissues in Triton alpestris*

In each column the first figure gives the number of mitoses counted, the second the total number of cells counted, and the third (heavy type) the former as a percentage of the latter.

(1) Substance	(2) No.	(3) Host neural tube	(4) Host epidermis	(5) Induced neural tube	(6) Ectodermal thickening
Oestrone	F52b-1	41/501	6/466	63/862	8/356
"	F52b-10	12/421	6/475	13/623	2/25
"	F52b-12	16/681	8/398	17/497	3/252
4:4'-dihydroxydiphenyl	F40b-3	41/949	7/362	44/927	2/110
Styryl blue	F63b-6	28/728	3/537	31/570	9/286
"	F63b-7	24/768	6/365	29/741	3/159
"	F63b-9	26/688	4/358	25/651	9/374
"	F63b-13	28/724	9/413	57/1007	8/327
1:2:5:6-dibenzanthracene*	F38b-5	31/578	3/385	29/1034	13/267
"	F101b-9	39/731	6/430	41/702	4/87
"	F101b-1†		7/767		11/331
"	F101b-2†	14/385	2/581		3/32
"	F101b-7†	29/688	19/645		34/898
Glycogen‡	F131d-8		10/599	30/694	14/1115
Glycogen‡	E131d-10		12/647	25/890	25/636
Means of percentages		4.24	1.46	4.35	2.62

Significance can be tested by calculating t for pairs of means of percentages. The values obtained are:

$$t_2 = 6.158, n = 25$$

$$t_3 = 0.327, n = 22$$

$$t_4 = 2.967, n = 23$$

$$t_2 = 6.078, n = 25$$

$$t_3 = 3.207, n = 26$$

$$t_4 = 3.146, n = 23$$

Adopting $P < 0.01$ as a test of significance, all these differences are significant except that between columns 1 and 3, i.e. between the host neural tube and induced neural tube. The same result is obtained if the t 's are calculated from the numbers of mitoses counted instead of from the percentages.

* Sodium salt of the α - β endo-succinate derivative.

† No neural induction.

‡ Implantations of Glycogen-Evocator complex into isolated ectoderm (Heatley, Waddington & Needham, 1937).

Several authors have recently discussed the effect of growth rate on the process of determination. Svetlov¹ showed that no re-determination, that is to say no re-determination, is possible in the *Urodele* tailbud during its period of most rapid growth, although it occurs later. He therefore suggests that rapid growth may prevent the occurrence of determination, but his results have been denied by Münch.²

Other authors have put forward hypotheses which have almost the exactly opposite sense. Perhaps the most firmly based of such suggestions is that of Balinsky.³ In a careful investigation of the induction of limbs by nasal placodes implanted into the sides of *Triton* larvae, he showed that the actual inducing part of the graft is the most actively growing nasal epithelium, and that the induction involves a considerable increase in the mitotic rate of the induced mesoderm. From these facts he develops a hypothesis which really falls into two parts. In the first place, aligning himself with Child and the Axial Gradient theory, he supposes that the power of induction is intimately connected with a high "metabolic activity". In criticism of this, one can point out that inducing power is clearly not immediately dependent on metabolism at all, let alone on particularly high metabolism, since it can be shown by dead material. It is, however, more reasonable to suggest, as Balinsky apparently wishes to, that it is only in consequence of its high metabolism that the organisation centre is able to produce the organising substance in an active form. There is, as we have seen (p. 35), some evidence for this view.

The second part of Balinsky's thesis is that the essential feature of the process of induction is the raising of the metabolic activity in the tissue on which the organiser acts; the actual differentiation which occurs in the induced tissue is considered as a consequence of the elevated "metabolic rate". The difficulty with this part of the hypothesis is that the term "metabolic rate" is so vague that it is, in fact, used to comprise just those phenomena for which it is alleged to provide an explanation. Balinsky, and most other authors who argue on these lines, includes in it "die allgemeine Aktivität aller Lebensprozesse", and these include the processes of differentiation. For instance, we are told that the eye-cup, when it induces a lens, does so by increasing the activity of the induced ectoderm; but the only evidence which we have of this increased

¹ Svetlov, 1934.

² Münch, 1938.

³ Balinsky, 1937.

activity is the visible differentiation which sets in, and the hypothesis amounts to no more than the tautologous statement that the cause of the differentiation is (an increase in activity which is) the differentiation.

It is absolutely essential to replace such vague terms as "life processes", "metabolic activity", etc. by precise concepts, even if this means at first a restriction of interest to comparatively trivial aspects of development. The mitotic rate, which has been mentioned earlier, is one such concept. It is clearly only a part of what is referred to by "metabolic rate", but we can measure it precisely and enquire what are its relations to various processes of induction. In the particular case considered here, we find that induction of neural tube involves a considerable increase in mitotic rate. This increase is always greater for neural tissue than for the stimulated ectoderm which does not differentiate in a neural direction. From this we can conclude that the potential relative growth rate of neural tissue is higher than that of the tissue comprising the ectodermal thickenings. It would be unjustified, however, to draw the further conclusion that the high growth rate was the primary condition of which the other properties of neural tissue are merely consequences. On the contrary, the fact that the mitotic rate of induced neural tissue is the same as that of the host neural tissue strongly suggests that the mitotic rate is not the overruling factor, but is itself controlled by other conditions in the tissue; there is no reason to attach greater importance to it than to any of the other properties of neural tissue, such as the position of the nuclei in the cells, etc.

The method of origin of differences in growth rate has an interest for fields of enquiry which are not normally reckoned to come under the scope of embryology. It is clearly in the highest degree relevant to the study of the origin of cancer. Thus it has been said that "it seems that the problem of the origin of cancer may be resolved into the problem of the origin of variations in cells in general and of discontinuous irreversible variations of growth rate in particular".¹ Any general theory of such phenomena must be mainly an embryological theory, since embryonic development (including regeneration) provides nearly all the examples known of irreversible cellular variation; even the occurrence of a somatic mutation can have no effect on the cells until

¹ Lockhart-Mummery, 1936.

the mutated allelomorph changes the course of a developmental process. It may, however, be too optimistic to expect that the general theory of the origin of discontinuous cellular variation will solve the problem of the origin of cancer, since it is very plausible to suggest that carcinogenesis is a special case. It may equally be dangerous to erect general hypotheses on the basis of phenomena observed in the particular case of carcinogenesis. Thus Haddow and his collaborators¹ have shown that when carcinogenic substances are injected into young rats, an inhibition of growth is produced. Arguing that this inhibition is an essential part of the process by which these same substances are capable of inducing cancer formation, they advance the general hypothesis that "variants characterised by permanently increased growth rate... are produced not by the process of direct growth stimulation as might be expected, but appear as a sequel to a long-continued period of growth inhibition". The facts recorded about the growth rate of induced neural tissue make it clear that this hypothesis cannot be accepted in the general form in which it is proposed. If it were true, we should find signs of growth inhibition in the ectodermal thickenings from which the induced neural tissue has arisen. The exact opposite is the case. The growth rate of the ectodermal thickenings is greater than that of the surrounding ectoderm. Similarly Balinsky does not describe any evidence that a growth inhibition precedes the production of limb mesenchyme with a heightened growth rate. It may be that carcinogenesis is a special case in which a preliminary inhibition of growth is essential, but the rule can certainly not be generalised.

The Individuation of Growth.

Organisers also affect growth rate through their individuating functions. Thus the different regions of the embryonic neural tube have different mitotic indices, which are part of their regional characteristics. The individuation of growth rates is most strikingly shown, however, in the phenomenon of regeneration. If the distal part of the limb of an amphibian is removed, a regeneration bud forms on the stump by the accumulation of small rapidly growing cells. These cells seem to be, to a considerable extent, indifferent and undetermined. Weiss² has reported that after transplantation

¹ Haddow & Robinson, 1937; Haddow, Scott & Scott, 1937.

² Weiss, 1927.

a regeneration bud from a limb may be caused to develop into a tail; and Schotte¹ claims that cells from a regenerating tail may be induced to form a lens. These results have not met with complete acceptance, but there seems no doubt that the fate of a regeneration bud is controlled by the stump to which it is attached; it forms just so much of the organ as is missing, and no more, and this could hardly be the case if the stump was without any influence on the course of events. The stump, in fact, controls the total amount of growth which is carried out by the cells of the bud; and if the stump is itself growing, the regeneration bud at first grows more rapidly until it catches up, and then slows down so that its growth keeps pace with that of the organ to which it is attached.

In any organ which can behave in this way, there must therefore be powerful influences which can control the growth of regenerating cells. It would be in the highest degree interesting to know whether these influences could control the growth of rapidly proliferating non-regenerating tissues, in particular cancer tissues.² But it is exceptionally difficult to test this, since very few, if any, cancers are known in organs which can regenerate.³ This in itself is not without interest, and is perhaps some evidence that in such organs the growth-controlling influences are powerful enough to overcome any tendency for the appearance of rapidly growing disorganised tissues.

Attempts have, however, been made to investigate the same problem in a slightly different way. Schotte⁴ has implanted young rat embryos into rat tumours and reports the appearance of tubular structures which he interprets as being induced by the grafted organiser. It is not clear, however, that these tubules can be regarded as individuated, i.e. as orderly, controlled structures; they may represent merely an evocation of a new histological tissue which remains disorderly in gross morphology; they may even be derived from the graft rather than from the host tissues. It seems in general rather unlikely that a small embryo, whose own structure would be considerably distorted by being inserted into a tumour, could control and reduce to order the tissues surrounding it. Perhaps a better chance of success would be expected if small pieces of tumour tissue are introduced into a larger organiser. This experiment has been made by Waddington

¹ Schotte & Hummel, 1939.

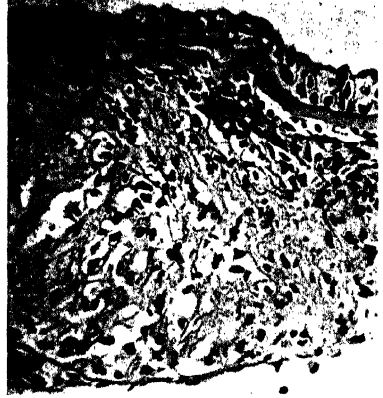
³ Needham, 1936 *b*.

² Waddington, 1935 *b*.

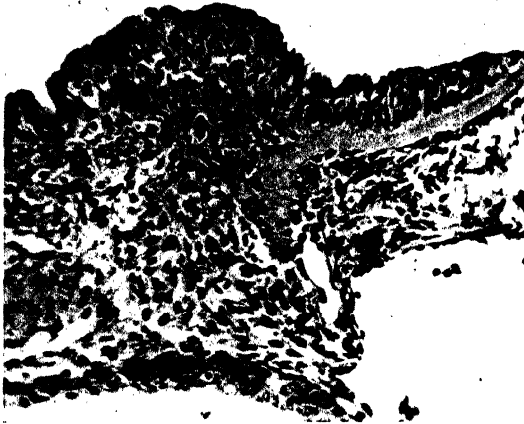
⁴ Schotte, 1938.



1



2



3

Regeneration buds from Axolotl limb transplanted into the body cavity of Salamander larvae. (1) A 6 day bud, fixed 21 days after transplanting, has formed a small nodule of cartilage. (2) A similar bud has developed into a mass of fibrous tissue. (3) Another similar graft has formed a mass of rapidly proliferating cells.

and Schukoff (unpubl.). Fragments of a fowl sarcoma, after some days' cultivation *in vitro*, were implanted into holes in the primitive streak of chick blastoderms. After 24 hours' cultivation *in vitro*, the hosts had formed fairly normal embryos, into which the grafted cancer tissue was fitted. But although there was some morphological assimilation in these cases, this might have been due simply to the mechanical effects of the gastrulation movements of the host. Definite evidence of an individuating effect of the host would be found if the grafted tissue became segmented into somites, or if it broke up into individual cells which permeated the host structures while the latter remained normal in morphology, but so far no such evidence has been obtained. Perhaps the experiment should not in any case be taken too seriously, since it is probably too much to expect a significant influence of the embryonic organiser on adult tumour tissue; the competences involved are likely to be too violently dissimilar. But until tumours can be found in regenerating organs, there appears to be no other opportunity to investigate the effect of individuation on uncontrolled growth.

It is certainly in many cases an oversimplification to suppose that the only character in which tumour tissue differs from the normal tissue from which it was derived is in its higher growth rate. It may be that some change in the cells renders them immune to the growth-controlling agents even though their growth rate is not particularly high. But it is interesting to discover how far the behaviour of normal cells may be made to approach that of tumour cells merely by removing the growth control. If a young regeneration bud from a newt or Axolotl is transplanted into the body cavity of a much smaller animal, such as a salamander larva, the host is unable to absorb the graft, which continues to grow at a high rate. The cells remain "indifferent" in type, although if the graft is made from a slightly older, already partially determined bud, it will form muscle and cartilage. The indifferent cells derived from young grafts look not unlike what sarcoma cells might be expected to be in Urodeles, and they may even penetrate and infiltrate the body wall of the host. So far no metastases have been found, and until they are shown to occur, it is dangerous to consider these translocated regeneration buds as a kind of cancer, although there is no doubt that with the removal of the organising influence of the stump they become considerably more cancer-like in behaviour. (See Plate facing page 123.)

CHAPTER XI

DEVELOPMENTAL PATTERNS

The Genic Control of Pattern.

THE morphogenetic movements and growth processes described in the last two chapters mould the developing tissues into organs. We will not attempt, at this stage, to define an organ, but at least we may say that an organ has a definite shape. This shape is dependent on the kind and magnitude of the movements which brought it into being. The set of movements leading to the formation of the definite shape of an organ are among the influences which have been referred to as the individuating factors of the organ; they are the individuating forces, as opposed to the individuating chemical influences which determine the qualitative natures of the various tissues which make up the organ. The fact that these forces are integrated with one another throughout a certain volume of tissue makes it convenient to speak of an individuation field.

The character of the individuation field, that is, the shape of the organ, may be altered by influences which affect the morphogenetic movements. Many cases are known in which such alterations are produced by genetic factors. Some of the simplest relate to differential growth rates. After the primordial organ is laid down, it may expand unequally by differential growth. The demonstration by D'Arcy Thompson¹ of possible examples of this phenomenon when phylogenetic relatives are compared has become classical. Examples in which the differential growth rates can be shown to be under the control of genes are widespread; for instance the differences in proportions of many races of domestic animals are often the result of differential growth taking place right up to maturity. The actual progress of the differential growth has been most fully analysed in plants, where the expansion can be divided into a part due to cell multiplication and another part due to cell elongation.

Changes in shape of an already formed organ may be produced

¹ Thompson, 1917.

by agencies other than growth. At one period of development a *Drosophila* wing consists of a thin-walled bag which is tightly inflated by the internal pressure of the body fluid; this stage is followed by a contraction and deflation of the wing to form a thin blade. The contraction, and thus the final shape of the wing, may be affected by genes, for instance by *dumpy* in *D. melanogaster* and *Blade* in *D. pseudo-obscura*. The first usually produces a shortened wing with a concave distal margin, the second a long pointed wing. The primary effect seems to be a disturbance of the elasticity and contractility of the wing surface in different directions. These are probably very delicately balanced to produce the normal wing shape, and the effect of the genes does not seem to be a very strong one, since in some allelomorphs of *dumpy*, *dp*⁰² for instance, the abnormal effect is only produced in a certain percentage of the animals. The effect of *Blade* is also variable, since sometimes the wing, instead of being long and pointed, is shortened and rounded rather as in *dumpy*; wings of different types may occur on the same animal. (Fig. 12 A.)

Many other examples of genetically controlled changes of shape, due to factors other than growth rate, can be found in the literature. A well-known and well-analysed case is the shape of the central field in *Ephesia* wings, investigated by Kühn,¹ where genes are known which affect the final position attained by a determination stream of something essential to pigment formation.

Some genetic effects on shape are probably due to alterations in the relation between two or more morphogenetic movements. A probable example is provided by the genes in *Drosophila* which cause a scalloping of the wing margin. In these wings, the general pattern is perfectly normal except that certain regions seem to have been removed from the edges of the wing. Goldschmidt suggested that this is in fact literally true, but his evidence that a marginal degeneration actually occurs is not convincing, since it depended on the appearance of notches in the wing during the period when it is now known to be contracting after a stage of extreme distension; the gradual appearance of the notches is most simply explained as a direct result of the decrease in internal tension. The true explanation of the scalloping is probably that the line along which the wing is folded into the imaginal bud in the late larval stage is shifted in relation to the already determined wing

¹ Kühn, 1936.

margin, parts of which are therefore left out. If this hypothesis is true, the effect of the genes is to change the relative positions of the processes of wing folding and marginal determination.¹ (Fig. 12 B.)

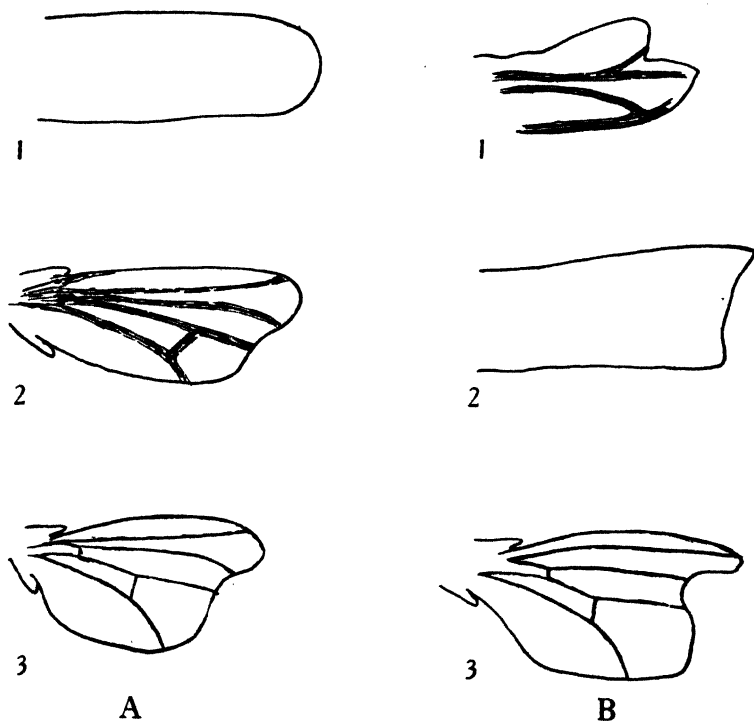


Fig. 12. Development of some wing mutants. A shows the development of *dumpy*; in 1 (beginning of true pupal period) the wing is a hollow sac, in 2 it is contracting and the veins are appearing as thick lines, in 3 the contraction is complete and the veins are narrower. B shows the development of *Xasta*; 1 is a prepupal wing, with the prepupal veins and a deep notch at the tip, 2 is the hollow sac stage, in which the distal notch is less obvious, and 3 is the fully contracted stage, in which the notch is again obvious.

In very many cases, pattern effects are caused by failures of the normal morphogenetic processes, these failures being themselves due to incomplete histological differentiation of certain tissues. The creeper gene in fowls is a well-known case. In these birds, there is failure of bone development in the long bones, probably

¹ Waddington, 1939c.

as a result of a depression of growth rate during a critical period,¹ which may in its turn be due to a failure of the blood system. Whatever the primary cause, the result of the incomplete histological development is a profound disturbance of all anatomical relations in the limbs. Similarly, the genetic abnormalities of venation pattern in *Drosophila* wings can be traced back to modifications of the normal histological processes which occur while the veins are being formed. In some cases, in which the effect on shape is complex, it can be plausibly suggested that the primary effect is on an inducing tissue. In rumpless fowls and tailless mice the final morphological changes which are observed are certainly the results of complicated processes in which induction plays some part. The genes affect the mesodermal, inducing parts of the vertebral axis as well as the neural regions, and it seems reasonable to suppose, as was done by Chesley² in his study of tailless mice, that it is the failure of the proper differentiation of the inducing mesoderm which is responsible for the other modifications which occur. Glücksohn-Schönheimer³ has shown that in tailless mice the phenomena are not so simple as might appear at first sight, since the tail is first formed and later cast off or re-sorbed. But even so she concludes that it is not unlikely that it is the mesoderm which is first affected. In Wright's⁴ otocephalic guinea-pigs, again, it is probable that the primary effect is an influence on the organiser, which brings in its train numerous secondary effects on the organs which should be induced.

Many of these genetic effects which involve organising tissues can be imitated by applying to the embryo abnormal conditions, such as dilute poisons, etc.⁵ Again, the evidence that these deleterious conditions affect primarily the organiser and only secondarily the other organs is in most cases not complete, but the facts certainly seem most easily explicable on that hypothesis. The analysis has not yet gone far enough for one to be able to identify with certainty the chemical systems affected by the external agents, and therefore presumably by the genes which are being imitated, but the line of work appears very promising.

We can distinguish at least three ways in which such deleterious conditions act. Firstly, they may specifically inhibit a particular

¹ Fell & Landauer, 1935.

² Chesley, 1935.

³ Glücksohn-Schönheimer, 1938.

⁴ Wright & Wagner, 1934.

⁵ Lehmann, 1936*a*, *b*.

region of the organiser, as in the experiments of Lehmann with lithium solutions. By exposure of amphibian embryos to these solutions at the appropriate times, it is possible to obtain defects such as cyclopia or otocephaly, which are closely similar to hereditary defects known in mammals, and it can be rather conclusively shown that the primary action has been exerted in the organiser. Similar effects are well known, but not so fully analysed, in other organisms.

Secondly, deleterious conditions might act as in Holtfreter's exogastrulation experiments in which the anterior end of the embryo is reduced in consequence of a failure of the anterior organiser to be invaginated. In this case it is not perfectly clear whether completely analogous effects are to be expected in mammals, which provide the most plausible parallels among the genetically determined abnormalities, since in the latter the invagination, at least of the mesoderm, is probably transverse to the long axis rather than along it. It may be that a similar inhibition of mesoderm invagination in mammals would lead rather to a *spina bifida* condition than to a malformation of the head. But it must not be forgotten that in this group we probably have to reckon, as in birds, with an organising action of the endoderm, which probably does invaginate in the direction of the long axis; the anencephalic embryos which Holtfreter¹ cites as parallels to his experimental amphibian embryos may be due to faulty invagination of the endoderm rather than of the mesoderm. But in either case the principle, of the absence of a certain region owing to the failure of its inductor to invaginate, remains the same.

As a third method of action of deleterious conditions, we may cite the reduction in mass of the invaginated material, which produces a dwarf but more or less complete embryo. This type of modification, which is well known in the chick, has recently been fully investigated by Holtfreter (unpubl.) in the Amphibia, where it may be caused by slight alcohol poisoning.

Patterns as Equilibria.

From all these examples we get the impression that a developmental shape is the result of a combination of many different processes. It is a "co-ordinative Einheitsleistung", in Lehmann's² phrase. In some sense, each definite form assumed by an organ

¹ Holtfreter, 1933*d*.

² Lehmann, 1933.

must be considered as representing an equilibrium between several opposing and interacting forces. The whole sequence of shapes during the development of an organ is a series of such equilibria. The picture is exactly the same as the one we derived of a gene reaction track when we were considering the development of substances; only in the present case each point on a track represents not a certain quantity of a substance but a certain configuration or arrangement of tissues.

Again, it is true, as we have just seen, that most gene substitutions merely disrupt the carefully adjusted harmony with which evolution has endowed the genotype. But in some cases here also we find genes which appear to shunt the developmental pattern into a new track which is almost as well defined as the normal one. Examples of both kinds of genes can be found affecting the legs in *Drosophila*.¹ In this animal the tarsus normally has five segments, whose lengths, although they show some variation, bear rather definite relations to one another. Certain genes disrupt this pattern. Eyeless-dominant, for example, causes a swelling of the proximal part of the tarsus, with some fusing of segments, and considerable disturbances of the hairs. More exaggerated effects of a rather similar kind are found in some aristopedia stocks, particularly those with ss^{ab} and ss^{as} , in which the legs are usually considerably shortened, and the proximal joints irregularly fused. Certain other genes, however, such as dachs d , four-jointed ff , and approximated app , while they also shorten the legs, do so according to a definite pattern; in all these stocks the tarsus has only four joints, which have definite relative lengths, and in addition in approximated there is a characteristic swelling of the penultimate joint. The compounds $d\ app$ and $ff\ app$ appear very like app , d and ff ; it seems that all three genes shunt development into a definite "four-jointed" track, and that the compounds also develop in this track. Compounds involving aristopedia, on the other hand, behave quite differently, showing great exaggeration of the shortening and fusion of the joints, so that in $ss^a\ d$, for example, the tarsus has almost completely disappeared; ss^a in fact appears to be a gene affecting the course of both the five-jointed and the four-jointed tracks. (Fig. 13.)

The mechanism of the leg segmentation is not fully known, but the first sign of the segments are a series of folds which appear in

¹ Waddington, 1940a, b.

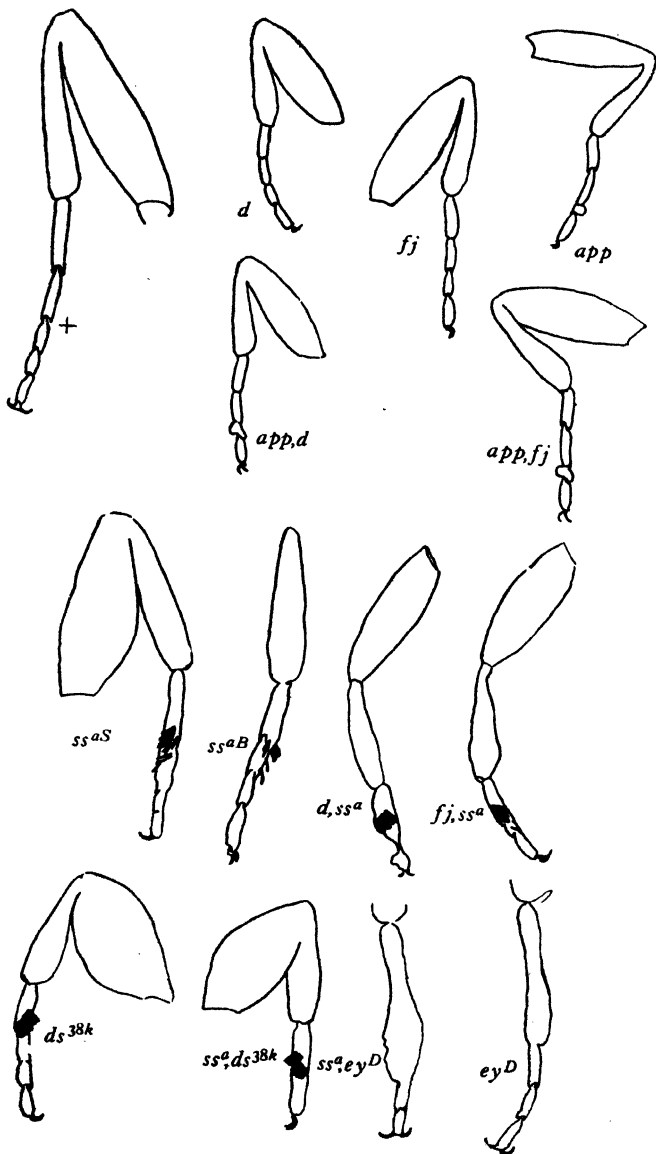


Fig. 13. Legs of *Drosophila*s containing the genes *dachs*, approximated, four-jointed, aristopedia, eyeless-dominant, dachsous-38k and various combinations of these. In the *ey^D* and *ey^D*, *ss^a* legs only the tarsus is shown. The hairs, except the sex combs, are omitted.

the leg rudiment while it is still a thick conical knob within the imaginal bud.¹ Presumably the factors determining the segmentation do so by influencing the mechanical properties of the tissue in such a way that the folds are shifted.

The fact that the tracks in a branching track system really represent equilibria, and should be pictured as the bottoms of valleys in a probability surface, is particularly clear in connection with the development of shapes, since it is here that we find the most striking evidence of far-reaching regulation after experimental disturbance. For instance, if pieces of ectoderm are added to or subtracted from an amphibian gastrula, a normal neurula is nevertheless formed. Clearly there is a certain equilibrium type of gastrulation movements, to which the altered system adjusts itself. This tendency to regulate to normal patterns seems to be considerably stronger than the similar tendency to form only certain definite sorts of tissues. At least it persists longer and can be found even in late stages of development and to some extent in the adult.

We have spoken of some pattern genes as causing merely a disruption of a pattern, others as shunting development into a new pattern track. This presupposes some criterion of what constitutes a new pattern as opposed to a modification of the normal one. Such a criterion is, however, extremely difficult to provide. In discussing the development of substances, the matter is fairly easily settled; in general a new developmental track means a new substance, and even if the substances involved are complex ones, such as legs and arista, we are not likely to have much difficulty in deciding what it is appropriate to call a new track and what a maldevelopment of the normal. But in connection with patterns we have no system of classification into qualitatively different kinds to guide us.

There is no doubt that some method of classifying patterns is desirable. During embryonic development, an animal certainly becomes more complex, and if we had a definition of what is meant by an alteration of pattern, we might be able to assess this increase in complexity in somewhat more quantitative terms. Needham,² for one, has discussed the attempts to measure the rate of differentiation, and pointed out the difficulties which are caused by the absence of any scale by which differentiation can be measured.

¹ Auerbach, 1936.

² Needham, 1936.

It is, however, when we turn from individual ontogenies to consider morphology from a comparative point of view that the need for a precise concept of pattern becomes most pressing. Comparative anatomists have in practice always employed some rather undefined concepts of this kind. It is recognised that a coelomate and a non-coelomate animal differ from one another qualitatively in a way which is not true of animals which exhibit merely variations of the coelomate type. The major groups of the animal kingdom are defined in terms of their pattern, although these patterns may appear in different forms in different members of the group. It is clear that in practice some changes in shape are considered to be changes in kind, while others are not. If this is so in comparative anatomy, that is another powerful reason why a system for dealing with the same matter should be worked out also for embryology.

The difficulties of providing a satisfactory system will probably be considerable, but there is no reason to suppose that they are insuperable, since a comparatively crude and unformulated system is in fact in use. The task of providing a suitable system should be one for topology. But according to the ideas of connectedness which, so far as I know, are the only ones now in use, all animals above the coelenterates reduce to annuli, and that is all that can be said about them. For biological purposes we shall undoubtedly need ideas of partial connectedness, which will allow one to make a distinction, for instance, between a simple intestine and one from which a gland has been budded off. Moreover, purely geometrical ideas do not appear to be adequate. For instance Wright¹ has described a race of guinea pigs in which the heterozygotes had pentadactyl limbs (the normal condition being three toes on the forefoot and four on the hindfoot) while in the homozygotes there were very numerous digits, up to eleven or twelve per foot. Now the difference in pattern between a pentadactyl and a four-toed condition is just the kind of pattern change which we wish to formulate precisely; but the difference between eleven and twelve toes is biologically much less important. Similarly, when an epithelium breaks up into a number of tubules, it is often a matter of indifference exactly how many such tubules there are. A biologically useful topology must find some way of getting round these difficulties of the purely formal approach.

¹ Wright, 1935.

Possibly this may be done by paying attention to another consideration which in any case cannot be neglected in dealing with animal pattern. We have pointed out several times that animal patterns are not static geometrical arrangements, but are dynamic, and we pointed out that the elements into which they must be resolved are movements. This is as true formally as it is for experimental analysis. Probably what we require for biological morphology is a system of topological operators, by the manipulation of which, in definite orders, the typical patterns can be built up.

For instance, consider the gastrulation of the chick. We have at first a flat plate of tissue, which we may represent as a plane F . At a certain point, the material sinks downwards, and then expands radially under the surface; we could represent this operation by the symbol $P \downarrow$, where the P stands for point. The next event is a similar sinking followed by expansion, occurring only in the upper layer, and centred along a line; we might represent this as $L \downarrow$. We then have a folding inwards of the upper layer along a line, to give the neural tube, and a folding upwards of the lower layer along a line to give the gut; we could represent these two $L \downarrow$ and $L \uparrow$. The whole developmental configuration, to the formation of the neural tube, could then be represented as follows:

$$F \begin{array}{c} P \downarrow \\ \cdot \\ L \downarrow \\ \cdot \\ L \uparrow \end{array}$$

If one made the same analysis of amphibian gastrulation, one would have to operate on a sphere, S . The sequence of operations would be

$$S \begin{array}{c} P \downarrow \\ \cdot \\ L \downarrow \\ \cdot \\ \cdot \end{array}$$

We may note the absence of the $L \uparrow$ operator on the lower layer, and clearly this is because an operation $L \downarrow$ on a closed surface such as a sphere gives a closed inner surface, whereas the same operation on a plane does not. Theorems could be derived which would exhibit such properties of the operational system.

This example is not, of course, offered as a serious attempt to formulate an adequate or appropriate set of symbols; it will have fulfilled its function if its inelegance annoys some mathematician sufficiently to provoke him to invent a better one.

CHAPTER XII

THE THEORY OF ORGANISATION

Fields.

PERHAPS the most striking feature of development is that it results in structures which we recognise as units: whole units which we call organisms, part units which we call organs. We have already seen that this involves phenomena of two different types. Each unit is built of several different materials, which must be formed by some chemical process, of which the evocator-competence reaction may provide a typical example. Further, these materials must be arranged into patterned structures, by processes of movement which we have also discussed.

As yet neither of these types of change is at all fully understood, and in most organisms we have hardly even a beginning of an analysis in such terms. For the preliminary consideration of such phenomena, which is what we are still mostly concerned with, it is useful to employ the concept of fields, which was introduced into embryology by Weiss¹ and Gurwitsch.²

This was essentially an advance in methodology; the field concept is not a causal principle, but a technique of analysis. The field theory can be used to describe any phenomenon which is extended in space-time. Thus we can speak of the diffusion field of the evocator surrounding an implant, or the field in which lens competence is present; and we might be able to discover the laws governing the variations of these entities throughout the field. This usage, however, in which the field concept is used merely to describe the spatial distribution of a single easily defined variable, is not a particularly valuable one; in fact, since it easily leads to confusion with the other and more valuable use, it should be avoided in favour of some more neutral term such as district or region.

The valuable use of the field concept is in connection with the whole complex of movements and other processes which lead to the formation of a developmental pattern. Thus the "lens field"

¹ Weiss, 1923.

² Gurwitsch, 1922.

should be taken to refer to those properties of a region of tissue by virtue of which the lens assumes a definite shape, with a certain configuration of lens fibres, etc. One can compare this biological usage with the usage in physics, where the field concept may be used, for example, to describe the course of the tubes of force in the neighbourhood of a magnet, or the distribution of potential near an electrically charged body. In these cases, we know the types of force, magnetic or electrical, which underlie the field. In the case of the lens field, we know much less of the nature of the underlying force, although we may suppose that it has something to do with the mutual repulsions and attractions of fibre-like lens proteins. But even if we do not know what the lens field is a field of, the field concept is a convenient way of expressing the fact that the underlying force is distributed in an orderly way which leads to the production of a structure with a definite and orderly pattern. It emphasises the fact that our aim must be to discover the spatially extended set of co-ordinated forces which work together to produce an organ. Moreover, many of our experimental treatments affect organs as complete units, causing some general alteration in shape which can best be described as a distortion of the field.

The circumstances in which the field concept is most useful are best seen in a definition given by Huxley,¹ who has done so much to elaborate and make use of it. "By a biological field-system, then, is meant a system which has the following characteristics. It is a spatial unity, in respect of certain properties at least. It is also an interrelated unity, in the sense that it may be deformed as a whole, and that, in regard to certain essential biological phenomena, events in one portion of the field have an important influence on events in other portions. It represents an organised whole with certain unitary activities, which must be studied as a unit, not merely as a summative resultant of its parts and their activities."²

As a very simple example of the use of the field concept we may consider the inheritance of fruit shape in squashes and gourds of the family Cucurbitaceae. Sinnott³ has shown that there are

¹ Huxley, 1935.

² An attempt to define the field in both philosophical and electrical terms will be found in Northrop & Burr, 1937.

³ Sinnott, 1935, 1936, 1939; Sinnott & Kaiser, 1934.

genes which act specifically on shape as opposed to size. Thus there are races with spheroidal, disc-shaped or club-shaped fruits. Each shape must be considered as a unity in the sense referred to by Huxley; it would be impossible to discover any regularity in the inheritance if we tried to analyse it in terms of single dimensions, such as length or breadth. The shapes are, however, so simple that in some cases it would be possible to contrast them

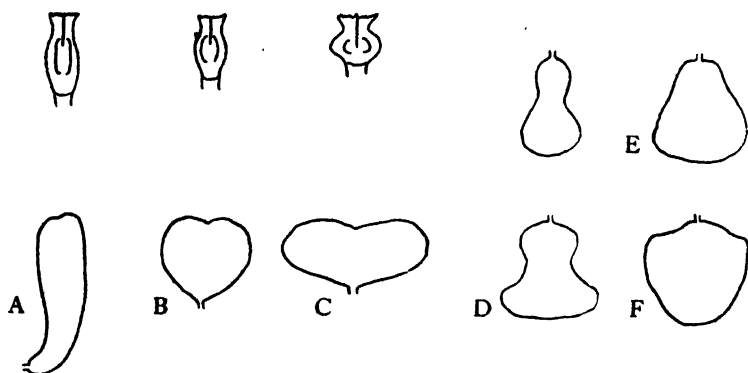


Fig. 14. Cucurbit fruits. A, B and C are three kinds in which the characteristic shape is already present in the very early stages, which are shown above. In D there is considerable change in shape during growth; the upper figure is a middle stage, the lower one a late stage. E and F are two fruits which differ in shape although in both of them the length is about equal to the width. (After Sinnott.)

by means of a relatively simple index, such as the ratio between length and breadth, although that would not be adequate to express the minor inheritable variations, such as that between *E* and *F* in Fig. 14. Moreover the fields must be considered as four-dimensional entities, since the fruits may change in shape during development. Sinnott shows that in many cases, such as *A*, *B* and *C* in Fig. 14, the definitive shape is attained very early, all subsequent development consisting merely in equal expansion in all directions. In other cases, however, such as that shown at *D*, the rates of growth in different planes remain unequal during the whole developmental period so that the shape is constantly changing.

The field concept has been used in this case simply in so far as we have considered moderately complex geometrical figures as single unities. It is perhaps tempting to go further than this, and to speak of the "club-shaped field" as controlling the growth of the fruit of the club race. But this is a very dangerous step. One must emphatically not consider the field as a sort of mould into which the fruit is poured; there is no agency standing outside the protoplasm of the fruit primordium and controlling the way in which it expands. The only justifiable point of view is to regard the field as a description of the consequences of the mutual interaction of the units of which it is composed. Thus if a lump of clay is shaken about it tends to assume a spherical form, whereas a pack of cards falls into a more pancake-like shape. Again, a floating droplet of a liquid whose molecules are more or less equidimensional has a spherical form, while if the molecules are fibrillar and attract one another the resulting mass is more elongated and forms a tactoid, such as we are familiar with in the example of the mitotic spindle.

The causally efficacious processes which determine the fruit fields must accordingly be looked for among the basic elements of which the fruit is composed. One might at first suspect that these basic elements were cells, and that the shapes of the fruits were due to the interactions between individual cells. However, Sinnott has shown that this is very unlikely. The growth of the fruit seems to be more or less independent of cellular events, since in the early stages of development it is mainly due to cell multiplication and in the later stages to cell expansion, but all the while it continues on an even course which shows no break at the time when the former process is giving way to the latter. One must conclude that the fruit should be analysed, in connection with its growth, not into cells but into basic elements of some other kind. The mere fact that plant cells can grow at different rates in their different parts¹ suggests that cells are not the right units in which to attempt to explain growth; but unfortunately we do not yet know what the correct units are.

The veins of a *Drosophila* wing provide an example of a field of rather a different kind: a pattern of lines lying in one plane. The pattern is quite simple. It consists of an anterior marginal vein (L_1) which is to some extent independent of the rest, four diverging

¹ Sinnott & Bloch, 1939.

longitudinal veins known as L_2 to L_5 , and two cross-veins, C_1 which runs between L_3 and L_4 , and C_2 between L_4 and L_5 . These four longitudinal veins and the two cross veins behave in

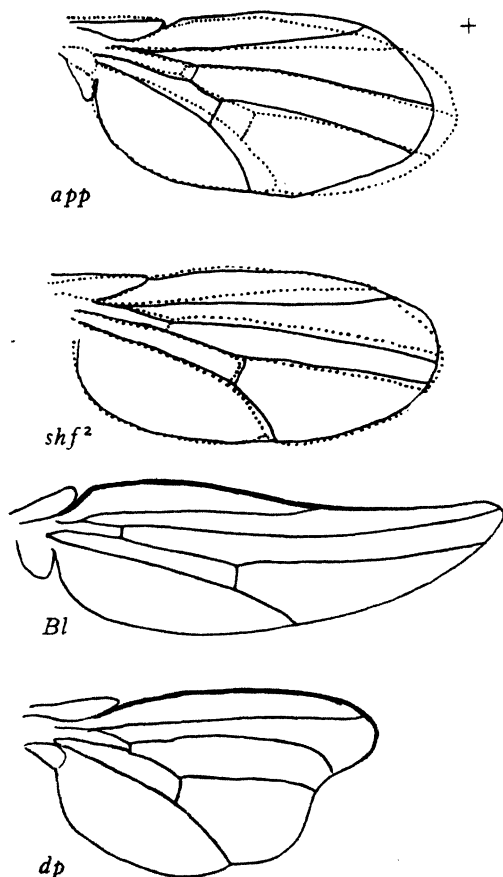


Fig. 15. Some mutant wings in *Drosophila*. The upper two figures show wings of approximated and shifted-2 drawn in full lines over the dotted outline of the normal wing. The third figure is Blade (from *D. pseudo-obscura*) and the fourth dumpy.

many respects as a single unit. For instance, the mutant shifted-2 has as its most obvious effect the bending downwards of L_3 towards L_4 ; but the effect is not really so localised, since L_2 also

bends down. The deformation, in fact, is a deformation of a whole region of the pattern, and not of an isolated vein. Similarly, mutants such as approximated appear at first sight merely to shorten the distance between the intercepts of the two cross veins on L_4 , but a closer examination again shows that the deformation is more general; in fact in this case it affects the entire venation, since all the veins diverge at a larger angle than normally. This causes C_1 to form more distally and C_2 more proximally than usual. The kind of forces involved are unknown, but one is reminded of the changes in mutual relations within a mass of soap bubbles when it is subjected to an external force. Finally the genes dumpy and Blade, which were mentioned earlier (p. 125) cause coordinated distortion of the entire venation, and we know something about the mechanisms by which this is brought about. The wings can be seen to be quite normal in their development until just about the stage when the veins are forming. At that time the wing rudiment is a hollow sac, which is collapsing to a flat plate and at the same time contracting in area; the veins appear as thickened ridges on the upper and lower surfaces. In dumpy it seems that the contraction is abnormally strong in the direction of the length of the wing, while in Blade it is exaggerated in the direction of the width. The distortion of the venation follows naturally if we consider the veins as being more rigid and less elastic than the wing surfaces, the marginal vein being the strongest. The field, then, can be partially analysed into its constituent elementary processes; we do not, indeed, know the forces which cause the appearance of the diverging veins, but for the rest we have to consider the contractility of the wing surface in different directions, and the elasticity of the partially formed veins, and both of these must eventually be expressed in terms of the physical properties of the cells concerned.

In all these examples, the value of the field concept has been that it emphasises the fact that several phenomena are coordinated with one another in such a way that they can all be grasped as a single whole; and it encourages one, when confronted with an apparently simple and isolated occurrence, to look more closely to see if this not really only the most striking member of a larger set. Its danger, on the other hand, is that it may seem to provide an explanation without the necessity of the detailed examination of the elementary processes concerned.

Complementarity.

Some authors would seem to dispute the idea that developmental fields can be analysed in this way. For if we speak of a limb field by virtue of which tissue within it forms a single unified limb, and if we could then state how the limb field arose, and how its properties could be derived from those of its ultimate elements, we should in effect have discovered the nature of the organisation of the limb. Now it is often stated that biological organisation is a primary fact which cannot itself be analysed. Such opinions may sometimes depend on no more than uncritical intuition, when they need not be taken very seriously. But they take on considerable importance when they are put forward by authors such as Niels Bohr.¹ Physicists have been so successful in developing the theoretical side of their science, and have produced such a penetrating analysis of certain parts of nature, that their opinions are worthy of the greatest respect from biologists, whose own theoretical scheme is so much more primitive.

Physics, as is well known, has been driven to the conclusion that certain of our apparently simple and basic ideas are, in the last analysis, incompatible with one another. The best known example is provided by the concepts of position and velocity; if we attempt to specify precisely the position of a body, we cannot at the same time specify precisely its velocity, and if we are dealing with bodies of the order of magnitude of electrons, the errors may become appreciable. The difficulty is often expounded by means of a technical illustration, but it is more than a mere technical impossibility in the ordinary sense. No technical advance could solve it. The two concepts of position and velocity are so related that, even in theory, an exact determination of one precludes an exact determination of the other.

Two concepts related in this way are said to be complementary, and the relationship is spoken of as complementarity. Bohr and others have suggested that complementarity is a general principle, and in particular that it may exist between some of the standard biological concepts and those of physics and chemistry in which the analysis of biological phenomena is usually attempted. For instance, it has been stated that life may be complementary to physico-chemical analysis, since the latter can only be carried on

¹ Bohr, 1933.

after the animal has been killed. Even if this were accepted, it would not of course invalidate all causal analysis of living things; the greater part of the sciences of genetics and embryology, with which we have been concerned in this book, have been derived from living organisms. However, it is not at all obvious that there is any fundamental difference between those parts of genetics, such as the laws of inheritance, which can be studied in the living, and those parts, such as cytology, which are more often investigated in the dead. Further, the idea that all physical and chemical analysis involves the death of the organism is clearly untrue; most vitamin and hormone work is performed on the whole living animal, and most manometric work on living fragments, while with the development of the use of "labelled" isotopes, a considerable amount of metabolism can be studied directly; again it is not easy to see any difference in philosophical status between the biochemistry of the living and the dead.

In fact, although there are undoubtedly technical difficulties in performing a full physico-chemical analysis on biological entities without killing them, there is no theoretical reason why this should always be so. Of course, it probably always will be so, just as it is probable that man will probably never be able to observe the other side of the moon. But that is no reason to claim that the moon's obverse side is not material; and similarly we cannot claim, because of this inessential technical difficulty, that life and physics are complementary. This would only follow if we had a theoretical analysis of the concept of life which could be shown to be complementary to the ideas of physics and chemistry.

Biology has, in fact, no well-defined concept of life. Indeed the existence of intermediates between the living and the non-living, such as viruses, makes it very doubtful whether any useful purpose would be served by trying to derive such a concept.¹ Certainly I do not wish to make such an attempt here.

The biologist, inexperienced in the ways of thinking of which the notion of complementarity is a part, may perhaps be excused if he raises the question of what are the actual consequences of a statement that two concepts are complementary. Must one conclude that a dead wall of incomprehensibility stands across the path of future investigation? This would be a very disheartening

¹ Pirie, 1937.

conclusion. But there are some considerations which, to the outsider, seem to offer a hope that such pessimism may not be necessary. What the physicists have in fact discovered is not, apparently, that there is no rule or regularity in the behaviour of a particle in relation to velocity and position, or that the whole situation is one of complete indeterminacy. On the contrary, exact laws of the spatio-temporal behaviour of particles can be found. The point is that in these laws velocity and position are not independent parameters. The fact, however, that definite laws exist confronts us with two alternatives, either to retain our ideas of position and velocity, and admit an essential non-causal indeterminate element in nature, or to modify our concepts of position and velocity and retain our ideas of causality.

The latter course may seem rather difficult; the notions of velocity and position work so well for macroscopic events, and are so deeply ingrained in common-sense thinking, that, rather than lose them, it may appear preferable to turn our back on the notion of strict causation, which has in any case always borne the emotional odium of seeming to deny our cherished ideas of the freedom of the will. But it would appear that our attachment to the notions of position and velocity is after all mainly a non-intellectual one; there is no reason why concepts which are very appropriate at one level of size should be equally valuable in another range of magnitudes.

This, however, is a question for physicists and philosophers. If any demonstration of complementarity occurs in biology, the position will be much easier. Except in relation to psychology, our common-sense system of thought does not contain any ingrained biological notions which are at the same time so precise that we shall not be fairly easily persuaded to modify them.

Organisation.

A complementarity to the ideas of physics and chemistry is often alleged not only of life, but of the notion of biological organisation. Indeed, in recent years, there has been a tendency either to regard organisation as one of the irreducible fundamental bases of all biology, or to invoke it, as though it were a well-defined concept, to fill up any awkward gaps in a theoretical structure. The latter use is perhaps the more reasonable; but it is

obviously dangerous *without a clear idea of exactly what is meant by the term, and when an attempt is made to reach such clarity, I think it will be found that the scope of the notion, as an explanatory principle, is not so great as has sometimes been suggested. On the other hand, it does provide the key to an extremely valuable method of thought. But before it can be accepted as a guide, it requires more precise formulation than it has yet received, at least in scientific contexts.*

In the first place, it is clear that the fundamental notion is organisation, which is capable of quantitative variation, rather than organism, which is not. The degree of organisation of an entity is usually considered to depend on the degree to which the parts of the entity are dependent on the whole. Now it is immediately clear, but is rarely pointed out, that the degree of dependence will be different in respect of different properties of the parts. For instance, if we consider a certain mechanism, such as a motor-car engine, the parts such as the cylinders, pistons, etc. are not in any way existentially dependent on the whole; they can exist perfectly well in isolation from it. They are, however, highly dependent on it in a certain context, namely with reference to the functioning of an internal combustion engine. When we speak of the dependence of the parts on the whole we must always have in mind some particular context; thus the parts of an entity can be said to be dependent on the whole, in a particular context, if, in order to express the properties of the parts in that context, some reference to the whole is necessary. For example, the eye may be considered either with reference to the function of vision, or as developing entity; in the first context, the relations of the retina and lens are those of two bodies which have to be adjusted to one another in order to focus light, etc., while in the second their relations are those of an inductive reaction and the mutual adjustments of growth rates. There is obviously no reason why the internal co-ordination of the eye, or, to put it in the way previously used, the dependence of the parts on whole, should be the same in the two cases. The eye may, and at certain periods of its existence undoubtedly does, have very different degrees of organisation in these two contexts.

The purpose for which the concept of organisation is usually invoked is to form part of the theoretical system for dealing with phenomena which seem to involve the subjection of otherwise

self-sufficient parts to some overriding whole. For instance, one might be tempted to advance "the organisation of the eye-forming region" as an explanation of the formation of a normal eye after some experimental disturbance of its rudiment. Instead of the individual cells behaving independently, they are subordinated to a general influence which causes them, even after disarrangement, to form a single normal organ. This is often expressed by saying that the tissue can no longer be adequately regarded as a mere mass of cells, but has attained a higher level of organisation, in which the relevance of the whole organ to the constituent cells can no longer be disregarded.¹

In some ways this type of expression suffers from the same difficulties as we noted in discussing fields. Where does the organisation come from? And what is meant by a higher level of organisation? The second of these questions is the easier to answer. The statement made earlier that organisation must be defined with reference to some context provides the clue. A new level of organisation is in fact nothing more than a new relevant context. When it is said that an organ rudiment has a higher level of organisation, as a developing entity, than a mere mass of cells, what is meant is that some organ is relevant to the former, while the latter has nothing to do with an organ, either because it is not yet competent or because it has passed the regulative stage and reached a point at which its development is completely mosaic, each fragment differentiating on its own without any reference to the whole.

The question of whence a new level of organisation is derived is more delicate. In discussing the analogous question about fields, we stated that the field must be regarded as a product of the interaction of its parts. If we applied this directly to the problem of organisation we might seem to arrive at the conclusion that all levels of organisation are implicit in the levels beneath them, which constitute their parts; and that would lead to denial of any true arising of new contexts, and thus to a denial of levels of organisation. In fact, we should find ourselves in a vicious circle. We do, however, seem to be confronted with the fact, which is very difficult to deny, of new contexts and new levels of organisation. It does seem that we are thinking in terms which are in some ways at different levels when we think of genes and their immediate products, and of the mirror symmetries of reduplicated legs. Or,

¹ Woodger, 1929; Needham, 1937.

to take a more extreme case outside the present field, psychology is hardly relevant in connection with the chemistry of sugars.

We must, then accept the existence of different levels of organisation as a fact of nature. On the other hand, we cannot easily suppose that the arising of a new level involves the appearance out of the blue of completely new properties of the elements of which the organised unit is composed. But the way out of the dilemma is clear. When elements of a certain degree of complexity become organised into an entity belonging to a higher level of organisation, we must suppose that the coherence of the higher level depends on properties which the isolated elements indeed possessed but which could not be exhibited until the elements entered into certain relations with one another. For instance, we have suggested that it may be possible to explain the organisation of regions of embryonic tissue into organ rudiments by supposing that they contain some orientated protein microstructure similar to that of a liquid crystal. Such a microstructure is on a different level of organisation to that of ordinary molecules in solutions. It depends on the mutual attractions and repulsions of fibre-like particles, which cannot be exhibited unless the molecules first form elongated fibres which then come together in large numbers. But the existence of the fibre level of organisation is not accounted for in terms of the elementary molecules plus some entity of a higher level, such as an overriding field. Instead we account for the formation of the fibre field by enlarging our ideas about the elementary molecules to include the fact that they can polymerise into fibres, which will then attract and repel one another in certain ways. That is to say, a new level of organisation cannot be accounted for in terms of the properties of its elementary units as they behave in isolation, but is accounted for if we add to these certain other properties which the units only exhibit when in combination with one another.

According to this view, one cannot explain any peculiar behaviour by postulating the existence of a new level of organisation. The advantages of the concept are not explanatory, but simply that it provides a terminology in which it is easy to admit the recognition that phenomena do not present themselves as being all of the same kind. And this is certainly an important advantage. In its absence we are practically forced to argue that the phenomena of sociology and chemistry are not significantly

different, a point of view which cannot easily be made very plausible. The admission that different levels of organisation exist frees us from such preconceptions. We feel no conviction that, for instance, the behaviour of a mass of tissue must be explicable in terms of the properties of its isolated cells. Instead we hope that investigation of the tissue will reveal new data about the mutual interactions of the cells when aggregated in a mass. Our aim is not merely to explain the complex by the simple, but also to discover more about the simple by studying the complex.

Science and Philosophy.

A concept such as organisation is not truly a part of science. A statement that a certain entity is organised does not attribute to it any specific properties from which its behaviour in particular circumstances can be deduced. Organisation is a philosophical idea, that is to say, part of a scheme into which all phenomena can be fitted. As such, a full discussion of it falls outside the scope of this book. But it is important to note that the scientist should arrive at such philosophical notions at the end of a scientific discussion rather than at the beginning. The criteria of philosophical validity are difficult to define, but they certainly can only be stated in relation to the purposes to which the philosophy will be put. As far as the scientist is concerned, the prime purpose of a philosophy is to be inclusive of anything which he may discover in nature; and that presupposes a flexibility which makes it unlikely that the philosophy will dictate to him what his discoveries should be. The first thing is to find the facts and their inter-relations; it is only in the second place that they should be fitted together into a general scheme.

Many scientists, in fact, conclude that any explicit formulation of a philosophy entails such dangers of suggesting the answers to problems that it is better avoided. Unfortunately, experience only too often confirms this suspicion. Even so flexible and powerful a system as dialectical materialism can be, and has been, supposed to lead to scientific consequences which are either exceedingly improbable or at best debatable. It has been urged, on the general principle that two entities which come in contact must modify one another, that human genes must be affected by the environment in which their bearers develop; and from this a somewhat Lamarckian theory of the inheritance of acquired characters has been developed.

But such a deduction neglects entirely the possibilities inherent in another part of the same system, namely in what we have spoken of as different levels of organisation, which in the dialectical materialist system is referred to under the heading of "the transition of quantity into quality". If we admit fully the benefits which have been conferred on Soviet citizens by their economic and social systems, we may allow that their genes have been affected by these alterations in conditions. But the changes are on the economic and social levels, and it is on these levels, and not necessarily on the chemical level, that the genes are affected. Economic betterment is a matter of food and hygiene; and its effect on genes is on the way in which they react to such conditions during the development of the organism. Social betterment means a better education, a fuller understanding of one's fellowmen; it affects what one might call the social fitness of genes, the likelihood that their bearers are willing or allowed to transmit them to future generations. In both these types of interaction, it would be possible, on theoretical grounds, that the effects on the genes go so far as to alter their chemical structure, but there is no philosophical reason why they should necessarily do so; and the evidence is all in favour of the hypothesis that they do not.

This example shows that even the best philosophies may be very bad guides to scientific facts. But the slightly dangerous qualities of philosophy should not frighten scientists into neglecting it completely. A system of philosophical concepts is not, as we have seen, a ready-made set of pigeonholes. But it is something much more important, namely a way of thought. One of the best known half truths about science is that asking the questions is more difficult than answering them. Whether this is an exaggeration or not, asking questions is at least one of the essential phases of scientific activity. It is in connection with this function that philosophy is most important. A new question implies a new context, that is to say, the attempt to fit a phenomenon into a system which has not previously been applied to it. In many fields, particularly of biology, our understanding is so limited that important new questions can be formulated merely by applying systems of philosophy which were developed some generations ago. That is true, for instance, of Spemann's discovery of the organiser. But as one comes to study phenomena which are very different from those in relation to which the older philosophies

were invented, the earlier methods of thought become less able to grapple with the situation. The developmental side of biology—embryology, genetics and evolution—seems to be reaching a point where radically new types of thinking are called for. In such circumstances it would be very unwise to despise the newer philosophies such as dialectical materialism, which are framed particularly in relation to progressive changes, even if they have sometimes led people astray. Philosophy, besides coming in, as a system, at the very end of scientific endeavour, is not without its importance, as a manner of thinking, before the experiment begins.

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INDEX

- Alternatives, sharpness of, 47, 84, 88,
91, 92, 131
Autarchic, etc., 66
Autocatalytic reactions, 101
- Blood groups, 57
- Carcinogenus, 32, 117
Carcinogenesis, 120 *seq.*
Cataract, 54
Chorio-allantoic grafts, 8, 100
Coat-colours, 74, 75
Competence, 17, 41, 46
and genes, 52, 54
lens, 43, 44, 46, 48
loss, 47
and organs, 50
origin, 42, 46
Complementarity, 140
Cornea, 54
Cucurbitaceae, 135
Cytoplasm, 52
- Determination, 17
Developmental alternatives, 47, 81,
83, 129
Dialectical materialism, 146
Diffusion, 32, 64, 101, 112, 114
Dogs, 89
Dosage compensation, 73
Double assurance, 49
Drosophila, aristopedia, 79 *seq.*, 84, 88,
89, 129
Bar, 60, 66
bobbed, 60, 70
bristles, 60, 85
eye colours, 53, 59, 63, 64, 65,
76 *seq.*, 83
leg genes, 81, 129
shaven, 71
wings, 85 *seq.*, 125, 137
yellow, 66
- Eggs, 52, 97
enucleated, 52
Endosperm, 57
Enzymes, 56, 69
- Ephestia*, 63, 76, 78, 125
Epigenetic landscape, 45, 88, 91 *seq.*,
129
Evocation, 16
and cytolysis, 26
of extra material, 16
and growth, 117
indirect, 28, 33
of organs, 50, 94, 95, 103
Evocators, acids, 24
activation, 28, 35, 39
distribution, 33
doses, 30
dyes, 35
and genotype, 53
glycogen, 23, 37
hydrocarbons, 24, 30, 51
inorganic, 27
localisation, 50, 97
protein, 34, 37
vegetable, 33
Evolution, 47, 53, 84, 90, 92
of dominance, 71
- Fibrisation, 110, 111
Fields, 134 *seq.*
Forces, 108, 112
Fowls, creeper, 127
frizzle, 89
- Gammarus*, 69, 71
Gastrulation, 106 *seq.*, 131, 133
forces, 108
Genes, dosage, 70
lethal, 59
nature of, 58
products, 56
Gene-effects, interaction of, 65
localisation of, 63, 89
Growth, 117 *seq.*
differential, 124, 136
Guinea-pigs, 54, 74, 127
- Habrobracon*, 64, 66
Hormones, 64, 65, 89
- Indeterminacy, 140

- Individuation, 14, 94
 and cancer, 122
 and competence, 51
 Induction, negative, 45

 Lens, 44, 48, 51, 109
 Liquid crystals, 109
 Localisation, of evocator, 50, 97
 of gene-effects, 62, 89
 within organiser, 95, 99 *seq.*
Lymantria, 69

 Maternal inheritance, 63, 97
 Metabolism, and forces, 112
 and induction, 119
 of organiser, 35 *seq.*, 99, 113
 Mice, 65, 127
 Modulators, 103
 Monsters, 128
 Mosaics, 65, 67
 Mosaic development, 49, 55

 Neomorphs, 88
 Neural palisades, 23
 Neural plate, shape, 114

 Organisation, 140, 142 *seq.*
 Organisers, amphibian, 5 *seq.*
 bird, 7 *seq.*
 and cancer, 121 *seq.*
 dead, 20, 94, 96
 fish, 6
 mammals, 12
 metabolism of, 35 *seq.*, 99, 113
 precursor, 98
 properties, 14, 105
 secondary, 6, 12

 Patterns, 61, 106, 124
 alternative, 129
 classification, 131
 Pleiotropy, 89
 Pollen tubes, 58
 Potency, 41

 Rabbit, colour, 75
 organiser, 13
 yellow-fatted, 57
 Rat, cataract, 54
 lethal gene, 89
 organiser, 13, 122
 Regeneration, 46, 121, 123
 Regional determination, 14, 94,
 100 *seq.*

 Segmentation, of legs, 129
 periodic, 101
 Segregation, 41, 45
 Self-differentiation, 19, 99, 100
 Sensitive periods, 84, 102, 128
 Sex, 64, 90
 Spindle, 110, 137
 Squashes, 135
 Stimulus, 29
 Substance, 60
 Suckers, 15

 Topology, 132

 Ultra-violet, 22

 Vitamin A, 57

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